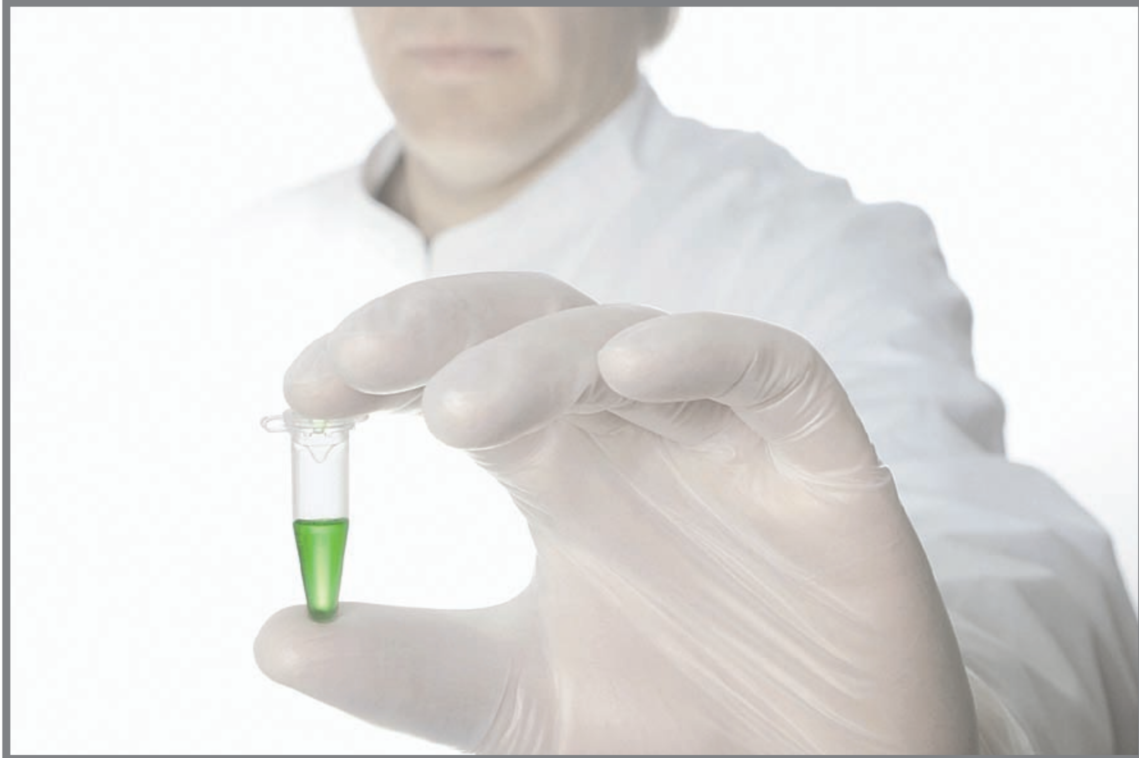
Forschungsbericht

2009



HPI

Heinrich-Pette-Institut für Experimentelle Virologie
und Immunologie an der Universität Hamburg

Leibniz
Gemeinschaft

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Inhaltsverzeichnis

Allgemeiner Überblick

Vorwort **7**
Wissenschaftliche Highlights **8**
Preise und Ehrungen **9**
Neubesetzungen **10**
Veranstaltungen **11**
Netzwerke **12**

Program Area "Virus-Host Interactions"

Introduction **14**
Research Projects **16**

Program Area "Cellular Dysregulation"

Introduction **38**
Research Projects **39**
I. Targeted genes and pathways of oncogenic transformation 39
II. Tumor suppressors and regulation of apoptosis, senescence and proliferation 44
III. Genomic instability and DNA repair mechanisms 49
IV. Epigenetic controls and chromatin organization 54
V. Immune cells: Targets of pathogenesis and therapy 57
VI. New tools for research and diagnosis 60

Veröffentlichungen **64**
Habilitationen, Dissertationen, Diplomarbeiten, Masterarbeiten, Bachelorarbeiten **68**
Die Kaufmännische Abteilung **70**
Institutionelle Förderung und Drittmittel 2009 .... **71**
Personalentwicklung 2009 .... **73**
Organigramm (Stand: Januar 2010) **75**

Allgemeiner Überblick

Vorwort

Der vorliegende Forschungsbericht informiert in kurzer und kompakter Form über Forschungsprogramme und wissenschaftliche Aktivitäten des Heinrich-Pette-Instituts im Jahre 2009. Wichtige Forschungsergebnisse werden ebenso vorgestellt wie aktuelle neue Entwicklungen an unserem Institut. Auf diese Weise will der Bericht – nicht zuletzt auch einer breiteren Öffentlichkeit – vermitteln, welche Fragestellungen unsere wissenschaftliche Grundlagenforschung prägen und welche Fortschritte wir im Jahr 2009 bei der Lösung drängender Probleme erzielen konnten.

Das HPI ist nun schon seit über 60 Jahren eine der ersten Adressen der virologischen und tumorbiologischen Grundlagenforschung nicht nur am Wissenschaftsstandort Hamburg, sondern deutschlandweit. Die wissenschaftliche Leistungsfähigkeit und sein Alleinstellungsmerkmal als einzige außeruniversitäre Einrichtung auf dem Gebiet der Virusforschung in Deutschland wurde dem HPI durch die externe Gutachterkommission der Leibniz Gemeinschaft in seiner Begehung 2009 erneut bestätigt. Wir nehmen dies mit großer Freude zur Kenntnis und sehen uns darin bestärkt, unsere anwendungsnahe Grundlagenforschung auf höchstem Niveau weiter zu betreiben und auszubauen.

Wir sind zuversichtlich, dass die Leistungsbilanz des vergangenen Jahres, die im vorliegenden Bericht dokumentiert ist, unsere Zuwendungsgeber, die Mitglieder des Kuratoriums und des Wissenschaftlichen Beirats, Freunde und Förderer des HPI und auch die Öffentlichkeit von der wissenschaftlichen und gesellschaftlichen Bedeutung des Heinrich-Pette-Instituts und seiner Forschungsarbeit überzeugen wird.

Hamburg, im Januar 2010

Prof. Dr. Thomas Dobner
Wissenschaftlicher Direktor

Wissenschaftliche Highlights

International vielbeachtete Studien zur Hemmung von HIV-Infektionen und zur Frage, warum sich bestimmte HIV-1 Stämme zu weltweiten AIDS-Pandemien ausbreiten konnten, gehörten im Jahr 2009 zu den wissenschaftlichen Highlights am Heinrich-Pette-Institut. Da HIV-1 hauptsächlich durch sexuelle Kontakte übertragen wird, ist es von weltweit großer Bedeutung, praktikable und kostengünstige Mikrobizide (Vaginalcremes) zu entwickeln, die die HIV-Infektionsrate drastisch reduzieren können. Wissenschaftler/innen des HPI gelang nun ein erster entscheidender Schritt in diese Richtung: Sie entdeckten, dass ein niedermolekularer Wirkstoff aus Grünem Tee, das Cachectin ECGC, sogenannte amyloide Strukturen in der Samenflüssigkeit hemmt und somit zu einer drastisch reduzierten Infektösität von HIV-1 bei der sexuellen Übertragung beiträgt (Hauber et al. 2009 Proc. Natl. Acad. Sci. USA). Hintergrund dieses Effekts ist die Tatsache, dass diese amyloiden Strukturen, SEVIs genannt, mit HIV-1 und der Zelloberfläche interagieren und dadurch eine erhöhte Infektionsrate bei der sexuellen Übertragung von HIV-1 ermöglichen. Mit der Entdeckung des natürlich in Grünem Tee vorkommenden SEVI-Hemmstoffes, so bestätigt Dr. Ilona Hauber (Abt. „Zellbiologie und Virologie“), erscheine jetzt die Entwicklung verbesserter und kostengünstiger Mikrobizide möglich, wie sie beispielsweise zur Prophylaxe in ressourcenarmen Ländern dringend benötigt werden.

Die HIV-1 Infektion des Menschen gehört zu den mehr als 200 bisher bekannten Zoonosen, bei denen ein Erreger erfolgreich aus dem Tierreich auf den Menschen übertreten konnte. Dafür musste das Virus verschiedene zelluläre Schutzmechanismen durch Mutationen und andere Anpassungsvorgänge überwinden. In einer gemeinsamen Publikation von Dr. Michael Schindler (Leiter der Nachwuchsgruppe „Virus-Pathogenese“) und Forschern der Universität Ulm konnte die Funktion verschiedener HIV-1 Proteine bei diesem Adaptationsprozess aufgekärt werden. Ein Vergleich unterschiedlicher HIV-1 Stämme mit Affen-Immundefizienzviren (SIV) zeigte, dass hierbei die viralen Proteine Vpu und Nef eine entscheidende Rolle innehaben (Sauter et al. 2009 Cell Host & Microbe). Die erfolgreiche Anpassung und hohe Infektiösität des HIV-1 M-Stammes beruht unter anderem auf der Funktion des Virusproteins Vpu. Vpu inaktiviert zwei zelluläre Schutzbarrieren auf Immunzellen, die auf der Expression von Tetherin-Molekülen und CD4-Rezeptoren beruhen. Im Gegensatz dazu sind die Vpu-Proteine der anderen HIV-1 Stämme entweder schwache Tetherin-Gegenspieler oder nicht dazu in der Lage, CD4-Rezeptoren auszuschalten. Bei SIV hingegen wird Tetherin durch das virale Nef Protein inaktiviert. Diese Befunde könnten erklären, warum sich nur der HIV-1 M-Stamm weltweit verbreitet hat und für die AIDS-Pandemie hauptverantwortlich ist.

Auch eine Studie der Abteilung „Molekulare Virologie“ könnte zur Entwicklung neuartiger niedermolekularer antiviraler Hemmstoffe führen – hier gegen Adenoviren. Dr. Orkide Koyuncu und Prof. Dr. Thomas Dobner beschrieben in der Fachzeitschrift *Journal of Virology,* wie die Funktionen des adenoviralen L4-100K-Proteins reguliert werden und wie sich dies auf die Virusvermehrung auswirkt. Das L4-100K (100K) Protein des Adenovirus Typ5 wirkt in der späten Phase der Virusinfektion – das ist die Phase, in der hocheffizient fast nur noch virale Proteine und Virusnachkommen produziert werden. Es vermittelt den Transport viraler mRNA und dessen Übersetzung in Virusproteine, es bindet an Grundbausteine der Viruskapsel, sogenannte Trimere, und transportiert sie in den Zellkern, wo sie später

zusammengesetzt werden. Die HPI-Wissenschaftler/innen zeigten erstmals, dass spezifische Bereiche im 100K-Protein durch Methylreste modifiziert werden. Diese, durch die zelluläre Methyltransferase PRMT1 katalysierte Reaktion, beeinflusst die Aktivität des 100K-Proteins und erhöht die Menge der Virusnachkommen (Koyuncu & Dobner. 2009 J. Virol.). Auf Grundlage dieser Erkenntnisse könnten erstmals neuartige experimentelle Therapien zur Behandlung von Adenovirusinfektionen entwickelt werden. Dafür sollen niedermolekulare Hemmstoffe der Methyltransferase PRMT1 identifiziert und getestet werden.

Preise und Ehrungen

Dr. Jan Chemnitz, Prof. Dr. Joachim Hauber und die Mitarbeiter des GO-Bio-Projektes (Abt. „Zellbiologie und Virologie“) gehörten zu den Gewinnern der aktuellen Wettbewerbsphase des „Science4Life Venture Cups 2009“. Mit diesem Preis wurden am 05. März 2009 in Berlin die besten Geschäftskonzepte von Gründern in den *Life Sciences* und der Chemie ausgezeichnet.

Prof. Dr. Joachim Hauber und Dr. Frank Buchholz vom Max-Planck-Institut in Dresden waren die Preisträger des „HIV/AIDS-Forschungspreises 2009“ der H.W. & J. Hector-Stiftung. Der mit 20.000 Euro dotierte Preis wurde am 25. Juni 2009 im Rahmen des Deutsch-Österreichisch-Schweizerischen AIDS-Kongresses SÖDAK2009 in St. Gallen verliehen.

Beim Deutsch-Österreichisch-Schweizerischen AIDS Kongresses (SÖDAK) in St. Gallen wurden ebenfalls erstmals Frauenforschungspreise verliehen. Die Arbeitsgruppe Ärztinnen und AIDS (3A), Europas größtes Netzwerk von HIV-Behandlerinnen, zeichnet mit dem Preis hervorragende Arbeiten von Wissenschaftlerinnen im HIV-Bereich aus. Den mit 1000 Euro dotierten „3A-Forschungspreis 2009“ erhielt Dr. Ilona Hauber (Abt. „Zellbiologie und Virologie“).

Dr. Gülsah Gabriel, Leiterin der Nachwuchsgruppe „Influenza-Pathogenese“, wurde am 09. September 2009 mit dem *„Influenza Award for Young Scientists 2009“* ausgezeichnet. Der mit 10.000 Euro dotierte Preis ist eine besondere Auszeichnung für Nachwuchsforscher auf dem Gebiet der Influenzaviren und wird jährlich durch die ESWI *(European Scientific Working Group on Influenza)* verliehen.

Allen Preisträgerinnen und Preisträgern sei an dieser Stelle nochmals herzlich gratuliert!

Das Heinrich-Pette-Institut freute sich über eine weitere Auszeichnung, die dem Institut im Jahr 2009 erstmals verliehen wurde: das TOTAL E-QUALITY Prädikat. Mit diesem Preis zeichnet der Verein TOTAL E-QUALITY bundesweit Firmen, Behörden und Einrichtungen aus, die sich durch ihre an Chancengleichheit ausgerichtete Personal- und Organisationspolitik ausweisen. Das HPI bekennt sich zu den „Forschungsorientierten Gleichstellungsstandards“ der DFG und verpflichtet sich damit, strukturelle und personelle Standards zu erfüllen, die eine Gleichstellung von Frauen und Männern in der Wissenschaft fördern. Die Verleihung des TOTAL E-QUALITY Prädikats ist eine willkommene Bestätigung auf diesem Weg.

Neubesetzungen

Neuer Wissenschaftlicher Direktor

Prof. Dr. Thomas Dobner ist seit Dezember neuer geschäftsführender Wissenschaftlicher Direktor des Heinrich-Pette-Instituts. Er löst Dr. Heinrich Hohenberg in seinem Amt ab, der in den letzten fünf Jahren im Vorstand des HPI tätig war, zwei Jahre als Stellvertretender und drei Jahre als Wissenschaftlicher Direktor. Damit konnte ein seit zwei Jahren eingeleiteter Generationswechsel im Vorstand des HPI vollzogen werden, der Teil einer umfassenden strategischen Weiterentwicklung ist. Die zukünftigen Aufgabenfelder des Wissenschaftlichen Direktors sieht Prof. Dr. Thomas Dobner primär in der Umsetzung wichtiger struktureller Veränderungen der Einrichtung, der Nachwuchsförderung und der Entwicklung eines gemeinsamen Forschungsleitbildes: „Der bereits eingeschlagene Weg in der wissenschaftlichen und strukturellen Neuausrichtung des HPI muss zielstrebig fortgesetzt werden, damit die wissenschaftliche Leistungsfähigkeit des Instituts und sein Alleinstellungsmerkmal als einzige außeruniversitäre Einrichtung auf dem Gebiet der Virusforschung in Deutschland ausgebaut werden.“ Das Heinrich-Pette-Institut dankt dem bisherigen Wissenschaftlichen Direktor Dr. Heinrich Hohenberg an dieser Stelle ausdrücklich für seine Verdienste um das Institut.

Neuer Kollegiumsvorsitzender

Mit Ernennung von Prof. Dr. Thomas Dobner zum Wissenschaftlichen Direktor des HPI musste im Dezember 2009 auch die Position des Kollegiumsvorsitzenden nachbesetzt werden. Diese wichtige Aufgabe wurde Prof. Dr. Joachim Hauber übertragen.

Neue Nachwuchsgruppe

Eine wichtige Säule der Nachwuchsförderung am Heinrich-Pette-Institut ist die Schaffung unabhängiger Nachwuchsgruppen, die durch exzellente, international anerkannte Nachwuchswissenschaftler/innen geleitet werden. Im Jahr 2009 konnte die mittlerweile dritte Nachwuchsgruppe „Influenza-Pathogenese“ unter Leitung von Frau Dr. Gülsah Gabriel installiert werden. Deren Forschungsschwerpunkte sind Mechanismen, wie Grippeviren von Vögeln auf Säugetiere und Menschen übergehen können und die Suche nach neuen Therapiemöglichkeiten gegen diese hochpathogenen Viren. Da hochpathogene aviäre Influenzaviren gelegentlich ihrem aviären Reservoir durch Überspringen der Wirtsbarriere entgehen und auf den Menschen übergehen können, stellen sie ein hohes pandemisches Potential dar.

Das HPI konnte die exzellente Nachwuchsforscherin von der Universität Oxford nach Hamburg holen. Bereits im Jahr 2005 entdeckte Dr. Gülsah Gabriel, dass genetische Veränderungen in einem Influenza-Protein, der so genannten RNA-abhängigen RNA-Polymerase, für eine erfolgreiche Adaptation von Vogelgrippeviren an Säugetierzellen verantwortlich sind. In ihrer aktuellen Publikation (G. Gabriel et al. 2009 Am J Pathol) analysierte sie dies gemeinsam mit Forschern aus Marburg noch genauer. Die Wissenschaftler/innen untersuchten zwei Virusstämme der aviären Influenza: einen an Säugetiere adaptierten und einen nicht adaptierten Stamm. Sie entdeckten, dass Mutationen im Gen der viralen

Polymerase für die erhöhte Infektiösität und Aggressivität des Virus im Mausmodell verantwortlich waren. Während der nicht-adaptierte Influenzastamm nur leichte Infektionen der Atemwege verursachte, führte der adaptierte Stamm zu einer gestörten Immunabwehr, schweren Lungenentzündungen, Infektionen des Gehirns und war tödlich. Bestimmte Mutationen in der viralen Polymerase erhöhten die Infektösität und die Menge von Virusnachkommen deutlich. Hier könnte eine neuartige Therapie gegen aggressive humanpathogene Grippeviren ansetzen.

In Anerkennung ihrer exzellenten Forschungsarbeit wurde Dr. Gülsah Gabriel 2009 in das „Emmy-Noether Förderprogramm" der Deutschen Forschungsgemeinschaft aufgenommen. Innerhalb des geförderten Projektes untersucht sie mit ihrer Gruppe die Rolle der zellulären Kernimportmaschinerie bei der Adaptation von aviären Influenza A (Vogelgrippe-) Viren an den Säuger. Die durch das „Emmy-Noether Programm" geförderten Studien sollen dazu beitragen, neue Determinanten für Wirtspezifität und Pathogenität zu ermitteln und eventuell zu neuen Strategien bei der Entwicklung von Therapeutika führen. Mit dem Stipendium werden für fünf Jahre insgesamt vier wissenschaftliche Stellen sowie erhebliche Sachmittel gefördert.

Veranstaltungen

HPI-Wissenschaftler/innen der beiden wissenschaftlichen Programmbereiche („Zelluläre Dysregulation" und „Virus-Wirts-Wechselwirkung") stellten im Rahmen eines Seminartags ihre Projekte vor und nutzten die jährliche Veranstaltung für intensive Diskussionen, Erfahrungsaustausche und die Entwicklung neuer Kooperationen. Zum Konzept der erfolgreichen Veranstaltungsreihe gehört außerdem, dass die Projekte überwiegend durch Doktoranden und PostDocs präsentiert werden, so dass junge Nachwuchswissenschaftler/innen wertvolle Erfahrungen vor einem großen Auditorium sammeln können.

Die „Pette-Lecture" ist seit 2006 der jährliche Höhepunkt der vom HPI ausgerichteten Seminarreihe mit renommierten nationalen und internationalen Wissenschaftlern. Im Jahr 2009 konnte das HPI Herrn Prof. Dr. Dr. h. c. mult. Harald zur Hausen (Nobelpreis der Medizin 2008) als Ehrenvortragenden begrüßen. Seine Einladung erfolgte in Anerkennung seiner herausragenden Verdienste um das Verständnis einer virus-bedingter Tumorerkrankung, des Zervixkarzinoms. Mit diesem herausragenden, international höchst anerkannten Virologen unterstreicht das HPI das hohe Niveau seiner Veranstaltungsreihe.

Im Rahmen der im Mai 2009 gestarteten „Leibniz Graduate School" fanden vier Intensivseminartage und Fortbildungen für Doktorandinnen und Doktoranden am HPI statt. Am 12. Oktober 2009 veranstaltete das „Leibniz Center for Infection" am HPI ein Fortbildungsseminar zum Thema „Antragstellung im 7. EU-Forschungsrahmenprogramm", das sich mit den *Starting Grants* für junge Wissenschaftlerinnen und Wissenschaftler beschäftigte. Weitere Themenschwerpunkte für Veranstaltungen waren der Umgang mit den Richtlinien zur *Good Laboratory Practice* sowie Konfliktmanagement und Präsentationstechniken.

Auch im Berichtszeitraum engagierten sich die Abteilungs- und Forschungsgruppenleiter als Organisatoren wissenschaftlicher Tagungen und prägten so den wissenschaftlichen Austausch ihrer Fachgebiete entscheidend mit. Hier einige Beispiele: Prof. Dr. Thomas Dobner veranstaltete vom 11. – 13. Februar 2009 den „2. Adenovirus-Workshop 2009" in Hamburg, an dem über 50 nationale und internationale Adenovirus-Forscher und Kliniker teilnahmen. Prof. Dr. Joachim Hauber war Koorganisator des Deutsch-Österreichisch-Schweizerischen AIDS Kongresses (SÖDAK, Juni 2009) in St. Gallen. Dr. Carol Stocking koorganisierte den EMBO-Workshop „RUNX transcription factors and development in disease", der vom 16. bis 18. August 2009 in Oxford, UK stattfand. Prof. Dr. Wolfgang Deppert organisierte den „12th Japanese-German Cancer Workshop" in Hamburg, bei dem seit 25 Jahren ein intensiver Austausch deutscher und japanischer Krebsforscher und Kliniker ermöglicht wird. Dr. Heinrich Hohenberg eröffnete am 11. November 2009 mit der Nikon GmbH das „Norddeutsche Imaging-Applikationszentrum" am HPI und veranstaltete ein Symposium sowie einen mehrtägigen Workshop „Bring Imaging to Life! Current Advances in Light Microscopy".

Zur 3. Nacht des Wissens in Hamburg am 07. November 2009 waren mehr als 2000 Besucher am HPI zu Gast und erlebten in vielen Mitmach-Aktionen und Vorträgen die „Faszination Virusforschung". Das HPI war an diesem Tag offizielle Passstation der Forschungsexpedition Deutschland 2009. Darüber hinaus besuchten 2009 mehrere Gruppen, u.a. Wissenschaftsjournalisten, Fachschaften verschiedener Universitäten, Hamburger Universitätsgesellschaft, Vertreter verschiedener Hamburger Behörden und Firmen, sowie mehrere Schulklassen das Institut.

Netzwerke

Leibniz-Center Infection (LCI)

Die im Jahr 2005 ins Leben gerufene Vernetzung des HPI mit den Leibniz-Instituten Forschungszentrum Borstel (FZB) und Bernhard-Nocht-Institut (BNI) im „Leibniz Center Infection" (LCI) konnte durch die Schaffung einer halben Koordinatorenstelle (seit 2008) und erfolgreich eingeworbene gemeinsame SAW-Projekte (Leibniz-Graduiertenschule, Computertomographisches Elektronenmikroskop) weiter ausgebaut werden. Auf verschiedenen Ebenen wurde eine stärkere institutionelle Vernetzung zwischen den LCI-Instituten mit dem Universitätsklinikum Hamburg-Eppendorf (UKE) initiiert und umgesetzt. Hier einige Beispiele: Gemeinsame Veranstaltungen der im Mai 2009 gestarteten Leibniz-Graduiertenschule „Modellsysteme für Infektionskrankheiten" und der unter UKE-Führung eingerichteten „Hamburg School for Structure and Dynamics in Infection" (SDI) fördern den wissenschaftlichen Austausch aller beteiligten Instititute. Gemeinsame Forschungsprojekte sowie die konkrete Planung einer Technologieplattform unter Beteiligung des UKE und des LCI werden eine noch engere Vernetzung ermöglichen. Mit dem Ziel einer verbesserten strategischen Ausrichtung auf zukünftige Forschungsnetzwerke, DFG-Initiativen, EU-Anträge oder Initiativen zur Stärkung der *Life Sciences*, aber auch der gemeinsamen Nutzung von so genannten *Core-Facilities* (Mauspathologie, *Imaging, Proteomics)* hat das HPI zudem den Dekan des UKE als Mitglied in das Kuratorium berufen. Seine Benennung wurde im Dezember 2009 vom Kuratorium beschlossen.

Landesexzellenzinitiative Hamburg

Im Januar 2009 hat die Stadt Hamburg eine eigene Landesexzellenzinitiative ins Leben gerufen, um Hamburger Forschungsverbünde unverzüglich und zielgerichtet zu fördern. Dabei werden die ausgewählten Projekte insgesamt 3,5 Jahre gefördert, wobei nach Ablauf der 1,5-jährigen ersten Förderphase alle Projekte zwischenbewertet werden. Nach einer positiven Evaluierung sollen anschließend erfolgsversprechende Projekte für weitere zwei Jahre, bis hin zur Entscheidung im Rahmen der Bundesexzellenzinitiative II, durch die Wissenschaftsstiftung Hamburg gefördert werden. Insgesamt ist das HPI sowohl an einem interdisziplinären Forschungsverbund (Exzellenzcluster „Nanotechnology in Medicine“/ NAME) als auch an einer Graduiertenschule („Hamburg School for Structure and Dynamics in Infection“, SDI) beteiligt.

Centre for Structural Systems Biology

Eine der am weitesten in die Zukunft reichende LCI-Aktivität in der norddeutschen Wissenschaftslandschaft betrifft die Beteiligung des LCI am Zentrum für strukturelle Systembiologie („Centre for Structural Systems Biology“, CSSB), das ab 2011 auf dem DESY-Campus geplant ist. Ziel dieser Großforschungseinrichtung mit infektiologischem Schwerpunkt ist das Erreichen eines möglichst umfassenden und detaillierten Verständnisses der diversen molekularen Wechselwirkungen innerhalb lebender Zellen und Gewebe im Rahmen struktur- und systembiologischer Grundlagenforschung. Es ist geplant, dass das LCI sich mit einer gemeinsamen Arbeitsgruppe an dieser interdisziplinären Einrichtung beteiligt. Das HPI engagierte sich beim Auftaktsymposium im September 2009, dem „1. International Symposium on Structural Systems Biology“, das von einem Konsortium unter Leitung von Prof. Dr. Christian Betzel (UKE, Hamburg) organisiert wurde und bei dem ca. 220 Forscherinnen und Forscher Themen der strukturellen Systembiologie diskutierten.

Program Area "Virus-Host Interactions"

Head: Prof. Joachim Hauber

Introduction

Viruses are obligatory parasites that can produce progeny only after successful infection of permissive host cells. Thus, virus replication depends on multiple and critical interactions involving viral and cellular components. Because viruses exploit cellular pathways, they also serve as valuable tools to understand the molecular basis of fundamental host cell mechanisms such as, for example, the regulation of immune responses or the induction of tumorigenesis. Therefore, the investigation of different viruses and their interaction with their respective host cell will not only improve our understanding of the different strategies that are applied by the respective viruses to secure progeny formation, but will also explicitly expand our knowledge on cellular metabolism. Finally, these studies will reveal novel viral and cellular target structures that may be subsequently exploited for the development of advanced antiviral therapies.

In 2009 a major research focus within the program "Virus-Host Interactions" was again on the functional analysis of viral regulatory/accessory proteins and their cellular binding partners. Prominent examples are studies on the Nef and Vpu protein of human immunodeficiency virus type 1 (HIV-1) or on various proteins encoded by the adenovirus early region. In case of HIV-1, these experiments not only revealed novel activities of these regulators but also provided new insights into how HIV modulates the immune response of the host. The studies on adenovirus regulatory proteins identified potential new cellular interaction partners, described novel mechanisms of how such viral factors are activated by posttranslational modifications and elucidated their activities with respect to the regulation of virus replication in more detail. Studies with Siman Virus 40 concentrated on the further investigation of the mode of action of the viral T antigen with respect to its interference with cell cycle control.

Another field of research concentrated on various aspects of tropism, virus spread and the establishment of virus latency. For example, studies concentrated on the receptor usage of XMRV, a xenotropic murine leukemia virus-related virus that infects humans and has been previously detected in prostate cancer patients. Other examples include the analysis of polymerase efficiency with respect to the systemic spread of influenza virus, or the contribution of Drosha, a cellular key enzyme in microRNA processing, to the maintenance of latency of Kaposi's sarcoma-associated herpesvirus (KSHV). Furthermore, a series of experiments with hepatitis B viruses focussed on mechanisms operational during virus entry and secretion or on experimental findings that suggest an unexpected modulation of the cellular transcription factor NF-AT by viral surface proteins. Functional analyses on the antiviral activity of cellular PML nuclear bodies provided novel insights into the restriction of Herpes Simplex-Virus 1 replication by host cell components.

The aforementioned investigations led in some cases also to projects that dealt with various aspects of current and future antiviral therapies. Examples mentioned here include the monitoring of the virological response after early antiviral treatment in hepatitis C virus (HCV) and HIV-1 coinfected subjects, the functional characterization of the first specific inhibitor of human semen-mediated enhancement of HIV-1 infection, and DNA vaccination studies aimed at the inhibition of de novo infection with avian influenza A virus.

Last but not least methodological developments, such as the generation of various fluorescence-labelled viruses in combination with time-lapse microscopy or cryopreparation techniques in combination with 3D electron microscopy allowed the initiation of several projects of critical virus-host cell interactions in realtime or at a "lifelike" ultrastructural level.

The significant scientific progress that was achieved in 2009 within the program area "Virus-Host Interactions" is reflected by numerous studies that were published in internationally recognized peer-reviewed scientific journals (listed at the end of this report). More detailed information on these and other projects can be extracted from the summaries following below or by directly accessing the respective publications. In sum, the projects presented here reflect in many ways state-of-the-art research in the field of "Experimental Virology", a field that represents the major research focus of the Heinrich-Pette-Institute.

Research Projects

A 49 kDa isoform of the adenovirus type 5 early region 1B 55K protein is sufficient to support virus replication

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The adenovirus type 5 (Ad5) E1B-55K protein is a multifunctional regulator of cell-cycle independent virus replication that participates in many processes required for maximal virus production. As part of a study of E1B-55K function, we generated Ad5 mutant H5*pm*4133 carrying stop codons after the second and seventh codon of the E1B reading frame, thereby eliminating synthesis of the full-length 55K product and its smaller derivatives. Unexpectedly, phenotypic studies revealed that H5*pm*4133 fully exhibits the characteristics of wild-type (wt) Ad5 in all assays tested. Immunoblot analyses demonstrated that H5pm4133 and wt Ad5 produce very low levels of polypeptides in the 48-to-49 kDa range, which lack the amino-terminal region but contains segments from the central and carboxy-terminal part of the 55K protein. Genetic and biochemical studies with different Ad5 mutants show that at least one of these isoform consists of two closely migrating polypeptides of 433 amino acid residues and 422 amino acid residues (R), which are produced by translation initiation at two downstream AUG codons of the 55K reading frame. Significantly, a virus mutant producing low levels of the 433R isoform alone, replicated to levels comparable to wt Ad5 demonstrating that this polypeptide provides essentially all functions required to promote maximal virus growth in human tumor cells. Altogether these results show that Ad5 infected cells contain several isoforms of E1B-55K and indicate that very low levels of proteins in the 55K reading frame are sufficient for a productive infection.

A flow cytometry-based FRET assay to identify and analyze protein-protein interactions in living cells

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Foersters resonance energy transfer (FRET) microscopy is widely used for the analysis of protein interactions in intact cells. However, FRET microscopy is technically challenging and does not allow to assess interactions in large cell numbers. To overcome these limitations we developed a flow cytometry-based FRET assay and analyzed dynamic interactions of human and simian immunodeficiency virus (HIV and SIV) Nef and Vpu proteins with cellular factors, as well as HIV Rev multimer-formation. Amongst others, we characterize the interaction of Vpu with CD317 (also termed Bst-2 or tetherin), a host restriction factor that inhibits HIV release from infected cells and demonstrate that the direct binding of both is mediated by the Vpu membrane-spanning region. Furthermore, we adapted our assay to allow the identification of novel protein interactions partners in a high-throughput format. The pre-

sented combination of FRET and FACS offers the precious possibility to discover and define protein interactions in living cells and is expected to contribute to the identification of novel therapeutic targets for medication of human diseases.

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A global analysis of evolutionary conservation among known and predicted gammaherpesvirus microRNAs

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MicroRNAs (miRNAs) are small, non-coding RNAs which post-transcriptionally regulate gene expression. The current release of the miRNA registry lists 16 viruses which encode a total of 146 miRNA hairpins. Strikingly, 139 of these are encoded by members of the herpesvirus family, suggesting an important role for miRNAs in the herpesvirus lifecycle. However, only a few cases of evolutionary conserved herpesvirus miRNAs have been described so far. We have performed a global analysis of miRNA conservation among gammaherpesviruses which is not limited to family members known to encode miRNAs, but also includes those which have not been previously analyzed. For this purpose, we performed a computational prediction of miRNA precursor hairpins in all fully sequenced gammaherpesvirus genomes, followed by combined sequence/structure alignments to identify potentially conserved candidates. Our analysis predicts that virtually all gammaherpesviruses encode miRNAs, most of which have not been described before. These miRNAs furthermore tend to be clustered at conserved genomic loci. However, only in two cases did we identify miRNAs which were also related in sequence: Rhesus rhadinovirus (RRV) is predicted to share at least 9 pre-miRNAs with the closely related Japanese Macaque Herpesvirus (JMHV), and 21 potentially conserved miRNAs were identified in the genomes of Rhesus lymphocryptovirus (rLCV) and Epstein-Barr Virus (EBV). All novel predictions of conserved EBV and rLCV miRNAs could be confirmed as bona fide latently expressed miRNAs, demonstrating the validity of our approach. Our analysis suggests that the evolutionary diversity of viral miRNAs reflects the requirement to mantain complementarity to target sequences, e.g. rapidly evolving sequences in the 3'-untranslated region of host transcripts. Knowledge of the full complement of conserved and non-conserved miRNAs encoded by rLCV and EBV will be helpful to identify relevant targets of miRNAs encoded by these oncogenic herpesviruses.

Adenovirus type 5 early region 1B 55K protein is phosphorylated by the alpha subunit of Casein Kinase 2

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Previous studies revealed that the adenovirus type 5 (Ad5) early region 1B 55K protein (E1B-55K) is subject to phosphorylation at serines 490, 491 and threonine 495. Here, we show that E1B-55K wild-type interacts with CK2alpha, whereas a phosphorylation-deficient E1B-55K

mutant protein where residues 490, 491 and 495 were changed to alanines (H5*pm*4174), exhibits severely reduced or no interaction. In addition, in vitro phosphorylation assays revealed that wild-type E1B-Glutathion-S-transferase constructs are readily being phosphorylated by the alpha subunit, but not by the CK2 holoenzyme. Furthermore, immunofluorescence analyses demonstrated a redistribution of the alpha catalytic subunit during wild-type- and H5*pm*4174-infection to the proximity of viral replication centres in the nucleus as well as distinct cytoplasmic accumulations. Consistent with previously published data, the phosphorylation-deficient E1B virus-mutant shows diverse defects like no p53-degradation and reduced progeny virion production. In sum, our data show for the first time that CK2alpha interacts with and phosphorylates Ad5 E1B-55K at S490/491 and T495 and that this regulation is necessary for efficient viral protein functions.

Analysis of adenoviral E1BN proteins

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The human adenovirus type 5 (Ad5) early region 1B (E1B) encodes at least five proteins. While it is established that E1B-55K and E1B-19K fulfill essential functions for viral replication and adenovirus mediated cell transformation little is known about E1B-156R, E1B-93R and E1B-84R. These proteins are produced from alternatively spliced forms of the major E1B-mRNA and encode the same 79-residue amino-terminus as E1B-55K, wherefore they are termed "E1BN proteins". As it is intriguing that Ad5 encodes at least four different proteins that share the same amino-terminal sequence, we set out to investigate the structural features of this element and the degree to which production of E1BN proteins is conserved among human adenoviruses. Due to studies of different human adenoviruses we could show, that the production of E1BN protein encoding mRNAs is not as limited as formerly suspected, but that it is conserved among all seven human adenovirus species. To now investigate the structure of the common amino-terminus, we selected Ad5 E1B-93R as a model. We could show by hydrodynamic, spectroscopic and NMR techniques, as well as computational analysis that E1B-93R is an intrinsically disordered protein (IDP). This further indicates that the same is true for E1B-84R and that the common amino-terminus might be an intrinsically disordered domain (IDD) within E1B-156R and E1B-55K. This assumption is further supported by computational models for structure and order/disorder content of E1B-55K, E1B-156R and E1B-84R. As intrinsic disorder is often found in proteins/domains that mediate multiple protein/protein interactions, this finding could lead to a better understanding of the functions of the E1BN proteins as well as E1B-55K. The widespread conservation further emphasizes the potentially important function of E1BN proteins.

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Arginine methylation of human adenovirus type 5 L4-100K protein is required for efficient virus production

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The adenovirus type 5 (Ad5) L4-100K non-structural protein (100K) mediates inhibition of cellular protein synthesis and selective translation of tripartite leader (TL) containing viral late mRNAs via ribosome shunting. In addition, 100K has been implicated in the trimerization and nuclear localization of hexon protein. We previously proved that 100K is a substrate of the protein arginine methylation machinery, an emergent post-translational modification system involved in a growing list of cellular processes, including transcriptional regulation, cell signaling, RNA processing and DNA repair. As understood at present 100K arginine methylation involves protein arginine methyltransferase 1 (PRMT1), which asymmetrically dimethylates arginines embedded in arginine-glycine-glycine (RGG) or glycine-arginine-rich (GAR) domains. To identify the methylated arginine residues and assess the role of 100K arginine methylation we generated amino acid substitution mutants in the RGG and GAR motifs to examine their effects in Ad-infected and plasmid-transfected cells. Arginine to glycine exchanges in the RGG boxes significantly diminished 100K methylation in the course of an infection and substantially reduced virus growth, demonstrating that 100K methylation in RGG motifs is an important host-cell function required for efficient Ad replication. Our data further indicate that PRMT1-catalyzed arginine methylation in the RGG boxes regulates the binding of 100K to hexon and promotes the capsid assembly of the structural protein as well as modulating TL-mRNA interaction. Furthermore, substitutions in GAR, but not RGG regions affected 100K nuclear import implying that the nuclear localization signal of 100K is located within the GAR sequence.

Conservation of Nef function across highly diverse lineages of SIVsmm

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SIVsmm is a simian immunodeficiency virus that persists efficiently without causing disease in naturally infected sooty mangabeys (SMs) but induces AIDS upon cross-species transmission to humans and macaques. Current phylogenetic data indicate that SIVsmm strains comprise a highly diverse group of viruses that can be subdivided into different lineages. Since only certain SIVsmm strains have successfully crossed the species barrier to humans and macaques, the question has been raised whether there are lineage specific dif-

ferences in SIVsmm biology. In the present study we examined whether representatives of five different SIVsmm lineages show differences in the function of the accessory Nef protein, which plays an important role in viral persistence, transmission and pathogenesis.

We found that *nef* alleles from all SIVsmm lineages down-modulated CD4, MHC-I, CD28 and CD3 and up-regulated the invariant chain (Ii) associated with immature MHC-II molecules in human-derived cells. Moreover, they generally suppressed the responsiveness of virally infected T cells to activation, enhanced virion infectivity and promoted virus replication in human peripheral blood mononuclear cells. The functional activity of these *nef* alleles in the various assays varied substantially between different strains of SIVsmm but quantitative analyses did not reveal any significant lineage-specific differences in Nef function. *Nef* alleles from different lineages of SIVsmm do not require adaptive changes to be functionally active in human cells. Strain rather than lineage-specific differences in Nef function may impact the virological and immunological feature of SIVsmm in SMs and possibly affected viral fitness and pathogenicity in human and macaque hosts.

Control of viral mRNA export by adenovirus regulatory proteins

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In adenovirus (Ad) infected cells regulated viral mRNA export and translation is controlled by at least three Ad proteins: E4orf6, E1B-55K and L4-100K. These directly or indirectly alter the stability of late viral RNAs and have been implicated in the preferential nuclear export of viral late RNA trans¬cripts, inhibition of cellular mRNA transport and protein synthesis, and selective translation of viral late mRNAs via *ribosome shunting*.

Efforts to identify functions that link E1B-55K, E4orf6 and L4-100K to viral late mRNA transport showed that each of the viral proteins possesses a single leucine-rich nuclear export signal (NES) of the HIV-1 Rev-type and can individually shuttle between the nuclear and cytoplasmic com¬part¬ment via the export receptor CRM1. To further investigate the role of CRM1-dependent nucleocytoplasmic shuttling for viral mRNA export and late gene expression, we generated and characterized virus mutants carrying amino acid exchanges in the NESs of E1B-55K, E4orf6 and L4-100K. In addition, virus growth was determined in the presence of the CRM1 inhibitors leptomycin B and CANc. Results from these studies confirm that efficient late Ad gene expression is dependent on CRM1. By contrast, however, preferential viral mRNA export occurs independent of intact NESs or active CRM1, demonstrating that viral mRNA export in Ad infected cells involves alternative pathways and export receptors. In fact, these data suggest substantial mechanistic differences between Ads and complex retroviruses in the control of viral RNA export and late viral gene expression.

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Crossing the species barrier: Role of Env-receptor interactions in gammaretrovirus infections

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The gammaretroviruses compromise a genus of exogenous and endogenous viruses that are widely spread in vertebrates. Analysis of endogenous retrovirus (ERVs) in the genomes of humans, mice, and other species indicates a longstanding association, probably dating back several hundred million years, during which retroviruses have repeatedly colonized host genomes. Phylogenetic studies of class I (gamma and epsilon) and class II (alpha, beta, delta and lenti) ERVs suggest that horizontal transfer of infectious virus between vertebrate classes occurs only rarely, although several important examples have occurred. In addition to the well-known jumping of a lentivirus from chimpanzees (SIVcpz) to humans (HIV-1), several recent reports have demonstrated the first bonafide human infection with a xenotropic MLV-related gammaretrovirus (XMRV). XMRV has been found in prostrate cancer patients and, more recently, at a high incidence in chronic fatigue syndrome. Using retroviral pseudotype assays and receptor binding assays, we have demonstrate that XMRV possesses a wide host range and efficiently infects feral mouse cells, as well as established human cell lines and primary cells. Interference assays confirm the classification of XMRV as a xenotropic MLV-like virus, which uses the transmembrane protein Xpr1 as a receptor. Interestingly, XMRV preferentially uses the human versus the murine Xpr1 variant, arguing that XMRV has inhabited the human population for a longer period.

Another example of cross-species transfer is the recent characterization of a Koala gammaretrovirus (KoLV), which is currently "invading" the host genome. This virus is closely related to the gibbon ape leukemia virus (GALV), although the vector responsible for transmission of the virus between these two species with distinct habitats has not been conclusively identified. We have recently isolated an ERV from *Mus cervicolor,* which we have dubbed McERV. McERV is closely related to the GALV and KoLV isolates and thus may share a common ancestor. Host and tissue spectrum analysis has shown that in contrast to most other gammaretrovirus isolates that use ubiquitously expressed membrane transporters as a receptor, McERV uses the myelin protein plasmolipin (PLLP) as a cellular receptor, whose expression is limited to kidney and brain. McERV-related isolates in *Mus musculus* show two different classes of env genes, suggesting a mechanism by which the virus has invaded other genomes. We are currently characterizing these defective genomes to determine their receptor usage.

Cryo-virology on the ultrastructural level

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The systemic combination of infected 3D culture cells or tissue systems, cryopreparation techniques and 3D electron microscopy (EM) are excellent tools for the analysis of "lifelike" virus- cell interaction at the ultrastructural level.

The best available approach so far to preserve the "lifelike" state of infected cells *in situ* is a chain of cryopreparation steps starting with freezing, followed by freeze-substitution, low-temperature embedding and polymerisation. In the case of virus-infected tissues or 3D cell culture networks, only high-pressure freezing permits adequate cryoimmobilisation up to a thickness of 200 µm that corresponds to approx. 40 cell layers of a 3D culture. Ultrathin sections of such samples, investigated by electron microscopy allow for the first time the structural analysis and 3D reconstruction of virus particles in a "lifelike" state in their cytoplasmic environment. It is accepted that cryopreparation techniques avoid the typical preparation artefacts induced by conventional EM-preparation techniques based on chemical fixation and total dehydration at room temperature. The conventional preparation steps change for instance the intracellular pH and the ion equilibrium, which affects the viral as well as the cytoplasmic structural elements, involved in the virus production and transport processes. Therefore a well-founded correlation between structural elements and functional aspects of virus production is difficult at least. Cryopreparation by contrast preserves the structural integrity of both, the viral and cytoplamic elements of infected cells or tissues.

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Daxx-mediated repression of adenovirus replication is counteracted by the viral E1B-55K protein

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The death-associated protein Daxx found in PML nuclear bodies (PML-NBs), is involved in transcriptional regulation and cellular intrinsic immune defense against incoming viruses. We found that knock-down of Daxx using siRNA techniques in a non-transformed human hepatocyte cell line results in significantly increased in adenoviral (Ad) replication, including enhanced viral mRNA synthesis and viral protein expression. This Daxx restriction imposed upon adenovirus growth is counteracted by early protein E1B-55K, a multifunctional regulator of cell-cylce independent Ad replication. The Ad protein binds to Daxx and induces its degradation through a proteasome-dependent pathway. We show that this process is independent of Ad E4orf6, known to promote the proteasomal degradation of cellular p53, Mre11, DNA Ligase IV and Integrin α3 in combination with E1B-55K. These results illustrate the importance of the PML-NBs associated factor Daxx in virus growth restriction and suggest that E1B-55K antagonizes the innate antiviral activities of Daxx and the PML-NBs to stimulate the viral replication program.

DNA vaccination with a single-plasmid construct coding for viruslike particles protects mice against infection with a highly pathogenic avian influenza A virus

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With seasonal outbreaks affecting millions of people each year and devastating pandemics, human influenza is a major health concern. The pandemic threat includes highly pathogenic avian influenza viruses (HPAIVs) that gained the ability to infect humans in Asia and quickly spread throughout the world. Major concerns have been raised regarding today's vaccine production systems against influenza viruses, and new strategies to design efficient vaccines are under intensive investigation. We demonstrated elsewhere that viruslike particles (VLPs) incorporating HPAIV hemagglutinin induce strong humoral immune response when injected in mice. In the current study, we evaluated a novel strategy that combines the immunogenicity of influenza VLPs and the advantages of DNA vaccines. We developed minimal expression vectors encoding all genetic information necessary to produce H7N1 influenza VLPs. We showed that mice vaccinated with small DNA amounts developed specific, high-titer neutralizing antibodies against homologous H7N1 strain and were protected against lethal doses of an antigenically distinct H7N7 HPAIV. Moreover, using some of these constructs, we were able to raise cross-neutralizing antibodies against an unrelated H5N1 HPAIV. DNA vaccination with constructs coding for influenza VLP production is a promising strategy to induce protection against different influenza viruses.

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Drosha is required for the maintenance of Kaposi's Sarcoma-associated Herpesvirus latency

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Kaposi's Sarcoma associated Herpesvirus (KSHV) is the etiological agent of several tumors, including Kaposi's Sarcoma (KS) and primary effusion lymphoma (PEL). The tumor cells in these malignancies are in a state of latent infection, a phase of the viral lifecycle during which viral gene expression is restricted to a small number of genes. While the molecular mechanisms of latency establishment are poorly understood, it has been hypothesized that viral miRNAs might contribute to the maintenance of latency via the silencing of lytic genes. We therefore have analyzed the effects of knock-down of two key enzymes involved in miRNA maturation on miRNA latency: Drosha, a RNAse which excises pre-miRNA hairpins from their precursor transcripts, and Dicer, which acts downstream of Drosha and further processes the hairpins to produce the mature miRNA duplexes. Indeed, shRNA-mediated knockdown of Drosha resulted in rapid and efficient induction of the lytic cycle in the PEL-derived cell line BCBL-1, with an efficiency that was comparable to treatment with chemical agents such as phorbol esters or histone deacetylase inhibitors. Surprisingly, however,

knockdown of Dicer did not result in a similar induction of the lytic cycle, thus indicating a role for Drosha in the maintenance of KSHV latency which is distinct from its function in the miRNA pathway. Recent data suggest that Drosha can directly cleave and destabilize mRNA transcripts which harbor pre-miRNA-like hairpins. By performig gene expression microarrays, we have identified several transcripts which are strongly upregulated in BCBL-1 cells upon Drosha inhibition and which bear hairpins that strongly resemble pre-miRNAs. The regulation of these transcripts by Drosha and their role during KSHV latency are currently under investigation.

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Efficient counteraction of tetherin by Vpu is required for HIV-1 replication in macrophages but not in ex vivo human lymphoid tissue

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The human immunodeficiency virus type 1 (HIV-1) Vpu protein degrades CD4 and counteracts a restriction factor termed tetherin (CD317; Bst-2) to enhance virion release. It has been suggested that both functions can be genetically separated by mutation of a serine residue at position 52. However, recent data suggest that the S52 phosphorylation site is also important for the ability of Vpu to counteract tetherin. To clarify this issue, we performed a comprehensive analysis of HIV-1 with a mutated casein kinase-II phosphorylation site (S52A) in Vpu in primary blood lymphocytes (PBL), macrophages and *ex vivo* human lymphoid tissue (HLT).

We show that mutation of S52A entirely disrupts Vpu-mediated degradation of CD4 and strongly impairs its ability to antagonize tetherin. Furthermore, casein-kinase II inhibitors blocked the ability of Vpu to degrade tetherin. Overall, Vpu S52A could only overcome low levels of tetherin and its activity decreased in a manner dependent on the amount of transiently or endogenously expressed tetherin. As a consequence, the S52A Vpu mutant virus was unable to replicate in macrophages, which express high levels of this restriction factor. In contrast, HIV-1 Vpu S52A caused CD4+ T-cell depletion and spread efficiently in *ex vivo* human lymphoid tissue and PBL, most likely because these cells express comparably low levels of tetherin.

Our data explain why the effect of the S52A mutation in Vpu on virus release is cell-type dependent and suggest that a reduced ability of Vpu to counteract tetherin impairs HIV-1 replication in macrophages, but not in tissue CD4+ T cells.

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Human adenovirus E4 region orf3/4 protein is required for efficient virus replication

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Human adenovirus early region 4 (E4) encodes at least six distinct polypeptides, defined as E4orf1 to E4orf6/7 according to the arrangement of the corresponding open reading frames. E4 gene products operate through a complex network of protein interactions with key viral and cellular regulatory components involved in transcription (E4orf6/7), apoptosis, cell cycle control and DNA repair (E4orf6), as well as host cell factors that regulate cell signaling (E4orf1), posttranslational modifications (E4orf4) and the integrity of PML-containing nuclear bodies (E4orf3).

As part of our studies of E4 function we generated mutant viruses carrying point mutations in the E4 genes. The phenotypic characterization of these mutants revealed that lesions affecting individual orfs had no or only moderate effects on lytic virus growth. By contrast, Ad mutants that lack the entire E4 region or fail to express the E4orf6 protein were severely restricted for growth. Unexpectedly, a virus mutant unable to express E4orf3 plus E4orf4 was as defective as the E4-minus and E4orf6 mutant viruses. More detailed genetic and biochemical analyses demonstrated that the E4orf3/E4orf4 double mutant exhibits a pleiotropic phenotype and fails to produce the E4orf3/4 fusion protein, which consists of the N-terminal 33 amino acid residues from orf3 and the C-terminal 28 amino acid residues from orf4. Consistent with this a virus mutant lacking E4orf3/4 and E4orf4 replicated to comparable levels as the E4-minus mutant. These results introduce a function for E4orf3/4 suggesting that E4orf3/4 and E4orf4 provide redundant functions for efficient viral replication, which is linked to their identical C-terminal regions. Further analysis of these mutant viruses revealed, that E4orf3/4 fusion protein might impact the early-to-late switch in the Ad5 lytic life cycle, thereby influencing viral DNA replication and late protein synthesis.

Identification of a new substrate of the E4orf6/E1B55K ubiquitin ligase complex

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The human adenovirus E4orf6 and E1B55K proteins promote viral replication by targeting several cellular proteins for degradation. The E4orf6 product has been shown by our group and others to form an E3 ubiquitin ligase complex that contains Elongins B and C and Cullin family member Cul5. E1B55K associates with this complex where it is believed to function primarily to introduce bound substrates for degradation via proteasomes. In addition to p53, its first known substrate, the E4orf6-E1B55K complex was shown to promote the degradation of Mre11 and DNA ligase IV; however, additional substrates are believed to exist. This notion is strengthened by the fact that none of these substrates seem likely to be associated with additional functions shown to be mediated by the E4orf6-associated E3 ubiquitin ligase complex, including export of late viral mRNAs and blockage of export of the bulk cellular mRNAs from the nucleus. In an attempt to identify new E4orf6/E1B55K substrates we undertook a proteomic screen using human p53-null, non-small cell lung carcinoma H1299 cells expressing either E4orf6 protein alone or in combination with E1B55K through infection by

appropriate adenovirus vectors. One cellular protein that appeared to be degraded by E1B55K in combination with the E4orf6 protein was a species of molecular mass ~130kDa that was identified as integrin alpha 3 subunit (Very Late Antigen 3 alpha subunit or VLA-3a). Preliminary analyses suggested that degradation may play a role in promoting release and spread of progeny virions.

Identification of novel interaction partners of E1B-55K and the E1BN proteins of human adenovirus type 5

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The human adenovirus type 5 (Ad5) early region 1B (E1B) encodes at least five proteins. Apart from the well known E1B-55K and E1B-19K three other proteins are produced from alternatively spliced forms of the major E1B-mRNA. They all encode the same 79-residue amino-terminus as E1B-55K (wherefore they are pooled as “E1BN proteins”) but differ in their carboxy-termini. Due to their number of amino acid residues they are termed E1B-156R, E1B-93R and E1B-84R. Today almost nothing is known about the functions of these proteins. Our aim is to identify their role in viral infection and cellular transformation. As a first step we set out to identify their cellular interaction partners via yeast two hybrid screens. Due to the common amino-terminus identified potential interactors of one E1BN protein might also interact with any of the others or with E1B-55K. In this approach we could identify 35 potential interaction partners. Among these were 8 factors that are involved in functions that are known to be modulated by E1B-55K namely DNA repair, antiviral defense and cell cycle control. While the confirmation of these potential interactors is ongoing, we could already identify one specific interaction partner of E1B-156R that does not interact with E1B-55K or the other E1BN proteins.

Inhibition of T-cell receptor-induced actin remodeling and relocalization of Lck are evolutionarily conserved activities of lentiviral Nef proteins.

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Nef, an important pathogenicity factor of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV), elevates virus replication in vivo. Among other activities, Nef affects T-cell receptor (TCR) signaling via several mechanisms. For HIV-1 Nef these include alteration of the organization and function of the immunological synapse (IS) such as relocalization of the Lck kinase, as well as early inhibition of TCR/CD3 complex (TCR-CD3)-mediated actin rearrangements and tyrosine phosphorylation. Although most SIV and HIV-2 Nef alleles (group 2) potently downregulate cell surface TCR-CD3, this activity was lost in the viral lineage that gave rise to HIV-1 and its SIV counterparts (group 1). To address the contribution of TCR-CD3 downregulation to Nef effects on TCR signal initiation, we compared the activities of 18 group 1 and group 2 Nef proteins, as well as SIV Nef mutants with

defects in TCR-CD3 downmodulation. We found that alteration of Lck's subcellular localization is largely conserved and occurs independently of actin remodeling inhibition or TCR-CD3 downregulation. Surprisingly, Nef proteins of both groups also strongly reduced TCR-induced actin remodeling and tyrosine phosphorylation on TCR-stimulatory surfaces and TCR-CD3 downmodulation competence by group 2 Nef proteins only slightly elevated these effects. Furthermore, Nef proteins from HIV-1 and SIV reduced conjugation between infected primary human T lymphocytes and Raji B cells and potently prevented F-actin polarization at the IS independently of their ability to downmodulate TCR-CD3. These results establish alterations of early TCR signaling events at the IS, including F-actin remodeling and relocalization of Lck, as evolutionary conserved activities of highly divergent lentiviral Nef proteins.

Localization and importance of the adenovirus E4orf4 protein during lytic infection

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The human adenovirus type 5 (Ad5) E4orf4 product has been studied extensively although in most cases as expressed from vectors in the absence of other viral products. Thus, relatively little is known about its role in the context of an adenovirus infection. Although considerable earlier work had indicated that the E4orf4 protein is not essential for replication, a recent study using *dl*359, an Ad5 mutant believed to produce a nonfunctional E4orf4 protein, suggested that E4orf4 is essential for virus growth in primary small-airway epithelial cells (C. O'Shea, et al., EMBO J. 24:1211–1221, 2005). Hence, to examine further the role of E4orf4 during virus infection, we generated for the first time a set of E4orf4 virus mutants in a common Ad5 genetic background. Such mutant viruses included those that express E4orf4 proteins containing various individual point mutations, those defective entirely in E4orf4 expression, and a mutant expressing wild-type E4orf4 fused to the green fluorescent protein. E4orf4 protein was found to localize primarily in nuclear structures shown to be viral replication centers, in nucleoli, and in perinuclear bodies. Importantly, E4orf4 was shown not to be essential for virus growth in either human tumor or primary cells, at least in tissue culture. Unlike E4orf4-null virus, mutant *dl*359 appeared to exhibit a gain-of-function phenotype that impairs virus growth. The *dl*359 E4orf4 protein, which contains a large in-frame internal deletion, clustered in aggregates enriched in Hsp70 and proteasome components. In addition, the late viral mRNAs produced by *dl*359 accumulated abnormally in a nuclear punctate pattern. Altogether, our results indicate that E4orf4 protein is not essential for virus growth in culture and that expression of the *dl*359 E4orf4 product interferes with viral replication, presumably through interactions with structures in the nucleus.

Localization determines function: N-terminally truncated NS5A fragments accumulate in the nucleus and impair HCV replication

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The Hepatitis C Virus (HCV) nonstructural protein 5A (NS5A) is an essential part of the ER-localized HCV-replicon complex. Although NS5A harbours a conserved NLS in its C-terminal domain, NS5A is associated with the cytoplasmic face of the ER by an amphipathic helix close to its N-terminus. METHODS: Intracellular distribution of NS5A in HCV replicating cells was analyzed by confocal microscopy and subcellular fractionation. The effect on HCV replication was analyzed using the JFH-1-based infection/replication system. RESULTS: During viral life cycle N-terminally truncated NS5A fragments are caspase-dependent formed that lack the ER-attachment signal and are localized within the nucleus. These N-terminally truncated fragments inhibit HCV replication. If their formation is blocked by inhibition of caspases HCV replication is increased. The C-terminal domain of NS5A binds to c-Raf and thereby localizes it to the replicon complex. This interaction is essential for HCV replication. The N-terminally truncated NS5A fragments are still able to bind c-Raf. However, due to their nuclear localization they withdraw c-Raf from the replicon complex into the nucleus resulting in an impaired HCV replication. CONCLUSIONS: Formation of N-terminally truncated NS5A fragments could represent a mechanism to regulate HCV replication by withdrawal of essential factors from the replicon complex.

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Manipulation of the cellular iron-uptake pathway by SIV and HIV Nef-alleles

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The HIV-1 Nef protein contributes to the damaging high levels of immune activation associated with T-cell loss and AIDS progression and is therefore commonly considered a virulence factor. However, we recently found that Nef-alleles derived from asymptomatic naturally simian immunodeficiency virus (SIV) infected monkeys and the less pathogenic HIV-2 block the responsiveness of virally infected T-cells to activation and may protect the infected host against damaging high levels of immune activation and loss of CD4+ T cells. Further analysis revealed that Transferrin receptor (TfR) levels were elevated in virally infected primary T-cells expressing Nef-alleles from SIV and HIV-2 but not from HIV-1. This effect was verified in 293T, THP-I and Jurkat T cells, as well as in infected primary lymphocytes and macrophages. Nef-expression alone was sufficient to induce upregulation of TfR. Nef-mediated inhibition of T-cell activation did not correlate with TfR upmodulation. Mutational analyses confirmed that both represent independent Nef functions and SIV Nef upregulates TfR by an N-terminal AP2-binding motif. Internalization experiments revealed that high TfR levels are a result of reduced receptor endocytosis following Nef expression. Strikingly, further experi-

ments showed that HIV-1 Nef but not SIV-Nef interacts with HfE, another regulator protein that is involved in cellular iron uptake. Our results show that differential Nef-mediated manipulation of receptors involved in cellular iron-uptake may contribute to the outcome of disease in lentiviral infections.

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Microscopical analysis of the HIV-1 life cycle in primary macrophages and cell to cell transfer to T-cells

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Macrophages are one of the major target cells for HIV-1 infection and play an important role in viral pathogenesis. Infected macrophages constitute a long-lived reservoir for HIV persistence and rebound. It has been shown that infected macrophages survive the infection for weeks and are a continuous source of ongoing virus production. Thus, HIV-1 infected macrophages pose a major challenge for HIV clearance from infected individuals.

The reasons for the longevity of infected macrophages are poorly understood. One possibility might be the special budding process in macrophages that seems to differ from that of other HIV target cells like CD4+ T-cells. In most cell types HIV-1 buds at the plasma membrane and new viral particles are released into the extracellular space directly after assembly.

In contrast to this, HIV-1 infected macrophages accumulate viral particles in large intracellular vacuoles that seem to originate from the endosomal pathway. Those particle accumulations are infectious over weeks and represent viral reservoirs which can not be eliminated by the immune system or by anti-retroviral therapies. Of note, those vesicles do not fuse with lysosomes because of lack of acidification and thereby the virions are not degraded. The mechanism underlying this defect in acidification is largely unknown. Additionally, it is matter of intense debate if those viral particle accumulations originate from direct budding into intracellular vesicles or are reinternalized virions, which have previously assembled at the plasma membrane. To address this question, we generated R5-tropic HIV-1 constructs carrying GFP in the GAG region and infected primary macrophages. Live-cell imaging is performed to track GAG and thereby assembly and budding of newly synthesized virions over time. Furthermore, we generated iGAG viruses with single knock-out mutations in nef, vpu, vpr and env. The latter constructs are used to investigate if acidification of lysosomal virus containing vesicles is prevented by one of these viral proteins and as a consequence allows HIV-1 to persist in macrophages.

Furthermore, we use this constructs to investigate cell to cell transfer by HIV-1 and the potential role of HIV-1 accessory genes on this route of virus transmission.

In sum, our experiments aim to elucidate the mechanisms of HIV-1 persistence in macrophages as well as the potential role of HIV-1 cell to cell transfer for virus transmission and spread. On the long term, our results will provide new information that is necessary to fight the HIV-1 macrophage reservoir in humans that poses a barrier towards eradication of the virus by antiviral therapies.

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Modulation of the nuclear transcription factor of activated T-cells by duck hepatitis B virus

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During infection with hepadnaviruses besides the infectious agent a high number of subviral particles without nucleocapsids are produced, which are able to change the infection dramatically. In addition, it was observed that the activation of the nuclear factor of activated T cells, regulated usually in cells of the immune system, was strongly influenced after infection. When primary duck liver cells were infected with purified virions of duck hepatitis B virus the activation of this factor was reduced in a similar way as it was achieved by inhibition of calcineurin, a cellular phosphatase necessary to control the factor, whereas the addition of subviral particles inhibited this reduction. It was found that the large surface protein of the virus was responsible for the reduced activity. Although this protein was embedded in similar amounts into the envelopes of both particles, only virions were able to inhibit the activity of the nuclear factor. An explanation of the different performances of the particles in primary duck liver cells apparently depends on the individual mode of insertion of the large surface proteins into the viral membrane. Furthermore, the nuclear factor of activated T cells could only be detected in liver sinusoidal endothelial cells, which was shown being attracted by virions but not by subviral particles.

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Monitoring the epigenetic fate of gammaherpesvirus genomes

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Latently infected cells enable herpesviruses to persist for the lifetime of their host and may also give rise to tumors, especially in immunocompromised individuals. The molecular processes which govern establishment and maintenance of latency, however, remain poorly understood. We have performed a comprehensive study of epigenetic changes associated with latency establishment by Kaposi's Sarcoma associated Herpesvirus (KSHV), a gammaherpesvirus which is etiologically linked to the development of several tumors, including Kaposi's Sarcoma (KS) and primary effusion lymphoma (PEL). For this purpose, we have developed a system to globally monitor the epigenetic status of viral genomes at high resolution, using custom-designed tiling microarrays in conjunction with immunoprecipitation of methylated DNA (MeDIP) and modified histones (ChIP).

Our analysis, for the first time, reveals highly distinct global DNA methylation patterns adopted by KSHV episomes upon the *de novo* establishment of latent infection. These patterns are non-random, as they do not reflect fluctuations of local CpG motif frequencies and are furthermore strikingly similar to those seen in several PEL-derived cell lines. However, such global CpG methylation patterns evolved in the course of weeks rather than days, suggesting that DNA methylation is unlikely to be sufficient for the primary establishment of latency. Instead, our data suggest that discrete histone modifications, especially widespread deposition of histones methylated at lysine 27 of Histone 3 (H3-K27), supress lytic gene

expression early during latency. Interestingly, many silenced lytic promoters simultaneously carried activating histone marks (e.g. acetylation of H3 lysine 9), thus bearing the hallmarks of transiently silenced ('poised') promoters which are typically seen in stem cells. Our finding thus indicate that KSHV exploits epigenetic host cell mechanisms in a highly specific manner to induce a metastable state of repression, which can be quickly reversed once conditions in the host cell become unfavourable for viral persistence.

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Production and characterization of human cytomegaloviruses carrying yellow fluorescent viral fusion proteins and application in antiviral screening

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Recombinant viruses labelled with fluorescent proteins are a useful tool in molecular virology. There are multiple applications for such viruses, e.g. studies on intracellular trafficking, protein localization or gene activity. We generated by homologous recombination three recombinant cytomegaloviruses carrying the enhanced yellow fluorescent protein (EYFP) fused with the viral proteins IE-2, pUL32 (pp150) and pUL83 (pp65). The characterization of growth kinetics showed that these viruses behaved like wildtype, even at low MOI. The expected fusion protein expression was detected by immunoblot and their localization was the same as for the unmodified proteins in wildtype virus infected cells. We established in vivo measurement of fluorescence intensity and used the recombinant viruses to measure inhibition of viral replication by neutralizing antibodies or antiviral substances. The use of these viruses in a pilot screen based on fluorescence intensity and high content analysis identified cellular kinase inhibitors that block viral infection. In summary, these viruses with individually EYFP-tagged proteins will be useful to study the dynamics of viral infection and antiviral screening in cell culture.

Selection and counterselection of the adenovir resistance mutation rtI233V during antiviral therapy

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Recently, we reported on three patients with chronic hepatitis B virus (HBV) infection who virologically failed under adefovir (ADF) therapy most likely due to the pre-existing HBV polymerase mutation rtI233V. Here, we describe two further patients with chronic HBV

infection who were found to develop the rtI233V mutation after initiating ADF therapy. These patients are the first cases known so far in which the rtI233V ADF resistance evolved under persistent HBV replication during HBV therapy with ADF. Interestingly, one of the previously described patients who was initially successfully switched from ADF to tenofovir (TDF) and became virologically suppressed subsequently, experienced a moderate but remarkable rebound of HBV-viremia after switching from TDF to entecavir due to the emergence of renal toxicity. Thus, we provide evidence for the selection and counterselection of the ADF resistance mutation rtI233V during antiviral therapy.

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Simian Virus 40 hijacks the ATR-Δp53-signaling pathway to override cell cycle control

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During infection, SV40 attempts to take hold of the cell while the host responds with various defense systems including the ATM/ATR-mediated DNA damage response pathway. Here we show that upon viral infection, ATR directly activates the p53-isoform Δp53, leading to up-regulation of Cdk-inhibitor p21 and accordingly down-regulation of S-phase promoting cyclin A-Cdk2/1 (AK) activity, thereby forcing the infected cell to stay in the replicative S-phase. Moreover, down-regulation of AK activity is a prerequisite for generating hypophosphorylated, origin-competent DNA polymerase α-primase (hypo-Polα), which is, unlike AK-phosphorylated Polα (P-Polα), recruited by SV40 large T antigen (T-Ag) to initiate bidirectional viral DNA replication. Preventing ATR-Δp53-mediated down-regulation of AK activity by inactivating the individual components of the ATR-Δp53-p21-signaling pathway significantly reduced the T-Ag-interacting hypo-Polα subpopulation and accordingly SV40 replication efficiency. In addition, the ATR-Δp53 pathway is also responsible for proteasomal degradation of the 180-kDa catalytic subunit of the T-Ag-non-interacting P-Polα, giving rice to the T-Ag-interacting hypo-Polα subclass.

Thus, the purpose of activating the ATR-Δp53-mediated intra-S checkpoint is to maintain the host in the replicative S-phase, an optimal environment for SV40 replication, and to modulate the host DNA replicase indispensable for viral amplification.

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Single Nef proteins from HIV-1 subtypes C and F fail to up-regulate invariant chain cell surface expression but are active for other functions

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The HIV-1 Nef protein plays a major role in viral immunopathogenesis, modulating surface expression of several immune receptors, altering signal transduction pathways and enhancing viral infectivity, among other activities. Nef also exhibits great intersubtype diversity

but most studies have only been focused on Nef proteins from the subtype B. Thus, little is known about the functional capacities of non-subtype B Nef proteins in host cells. We investigated cell surface regulation of MHC-I, MHC-II, the MHC-II-associated chaperone invariant chain (Ii), CD4, CD3 and CD28 in cells transfected or infected with five different Nef-alleles including one HIV-1 Subype C and F allele. No significant difference among the Nef proteins regarding CD3, CD28 and MHC-II downregulation was observed. The NefC showed a slightly, yet significant, diminished capacity to downregulate MHC-I in all cells, as well as to downregulate CD4 in Jurkat cells and PBMCs. Strikingly, the two alleles from NefC and NefF were unable to up-regulate the Ii chain both in transfected and infected cells. Moreover, internalization rate of surface Ii chain was slightly affected by NefC and NefF, whereas it was drastically reduced by NefB. Nef domains known to be involved in Ii chain up-regulation were conserved among the five alleles analyzed here. In summary, we identified two primary HIV-1 NefC and NefF-alleles that are selectively impaired for Ii-upregulation and which may help to elucidate the mechanism of this Nef function in the future. It will be important to determine whether the observed differences are HIV-1 subtype dependent and influence viral immunopathogenesis.

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Spread of infection and lymphocyte depletion in mice depends on polymerase of influenza virus

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SC35M is a mouse-adapted variant of the highly pathogenic avian influenza virus (HPAIV) SC35. We have previously shown that adaptation is mediated by mutations in the viral polymerase and that it is paralleled by the acquisition of high pathogenicity for mice (Gabriel et al., 2005). In the present study, we have compared virus spread and organ tropism of SC35 and SC35M in mice. We show that SC35 virus causes mild bronchiolitis in these animals whereas infection with SC35M virus leads to severe hemorrhagic pneumonia with dissemination to other organs including the brain. In SC35M infected animals, viral RNA and viral antigen were detected in monocytes and macrophages, and SC35M, unlike SC35, replicated in lymphocyte and macrophage cultures *in vitro*. SC35M did not induce an adequate cytokine response but, unlike SC35, caused severe lymphopenia in mice. These observations suggest that the high efficiency of the polymerase of SC35M is responsible for infection and depletion of lymphocytes and other white blood cells resulting in immune suppression and systemic virus spread.

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Sustained virological response after early antiviral treatment of acute hepatitis C virus

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Limited data exist describing the clinical outcome and immunological response primed during simultaneously acquired acute hepatitis C virus (HCV) and human immunodeficiency virus (HIV) coinfection. Here we presented detailed clinical and immunological analysis of 3 individuals after concomitant infection with acute HCV and primary HIV.

In addition to longitudinal clinical parameters, virus-specific T cell responses were assessed using Elispot, standard proliferative (carboxyfluorescein diacetate succinimidyl ester), and in vitro CD4+ T cell assays.

In all patients, anti-HCV treatment was started with pegylated interferon-α, and antiretroviral therapy was coadministered early during primary infection. HCV viremia was cleared under therapy with pegylated interferon-α in all 3 cases. In 2 patients, HIV replication was contained even after antiretroviral therapy had been interrupted, which was associated with strong HIV-specific CD8+ and CD4+ T cell responses. In these 2 patients, multispecific HCV CD4+ T cell responses could also be detected. No HCV-specific CD4+ T cell responses were detected in the third patient, who also had the lowest nadir CD4+ cell count during primary HIV infection (<200 cells/µL).

In sum, anti-HIV and -HCV therapy should be considered early in cases of concomitant acute HCV and HIV coinfection, because succcessful therapy of HCV viremia seems possible even during primary HIV infection. HCV-specific T cell immunity is generated during primary HIV infection and can be preserved by HCV treatment. However, the optimal treatment algorithm needs to be established in prospective, randomized trials.

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T-cell activation and LTR-transcription by lentiviral Tat proteins

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For productive replication HIV-1 needs to induce cellular activation and proliferation. Strikingly, elevated immune activation correlates with AIDS-progression in HIV-1 infected patients, whereas asymptomatic SIV and less pathogenic HIV-2 infections are characterized by low levels of chronic immune activation despite high viral loads. HIV-1 Tat mediates viral gene expression via binding to the TAR-element of the LTR-promoter and is also proposed to be the main inducer of T-cell hyperactivation. Therefore, we hypothesized that Tat-proteins may differ in their ability to activate T-cells and/or the LTR-promoter.

Methods: The LTR- and T-cell-activation activities of different *tat*-allels were analyzed in stable transfected HIV-1-LTR-Luc, SIV-LTR-Luc, NFkappaB-Luc, NFAT-Luc cell lines and primary T-cells.

Our results show that HIV-1 Tats and their SIVcpz-precursors induce transcription via the HIV-1- and the SIV-LTR, whereas SIV- and HIV-2-Tats only efficiently transactivate the SIV-LTR. Furthermore, all tested HIV-1, HIV-2 and SIV-Tats enhance NFkappaB- and NFAT-activation, thus contributing to T-cell-activation. Interestingly, low levels of HIV-1 LTR transactivation induced by SIV- and HIV-2-Tats correlated highly significant with their ability to induce NfkappaB.

These results suggest that Tat induced T-cell-hyperactivation does not significantly contribute to the levels of chronic and general immune activation observed during AIDS progression. Moreover, we propose that highly divergent lentiviral Tat-proteins evolved two independent strategies to transactivate the LTR. First, by direct binding to the TAR-element, as well as indirectly through induction of NFAT and NFkappaB transcription factors.

Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains

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Vpu proteins of pandemic HIV-1 M strains degrade the viral receptor CD4 and antagonize human tetherin to promote viral release and replication. We show that Vpus from SIVgsn, SIVmus, and SIVmon infecting *Cercopithecus* primate species also degrade CD4 and antagonize tetherin. In contrast, SIVcpz, the immediate precursor of HIV-1, whose Vpu shares a common ancestry with SIVgsn/mus/mon Vpu, uses Nef rather than Vpu to counteract chimpanzee tetherin. Human tetherin, however, is resistant to Nef and thus poses a significant barrier to zoonotic transmission of SIVcpz to humans. Remarkably, Vpus from nonpandemic HIV-1 O strains are poor tetherin antagonists, whereas those from the rare group N viruses do not degrade CD4. Thus, only HIV-1 M evolved a fully functional Vpu following the three independent cross-species transmissions that resulted in HIV-1 groups M, N, and O. This may explain why group M viruses are almost entirely responsible for the global HIV/AIDS pandemic.

The Hepatitis C virus non-structural NS5A protein impairs both the innate and adaptive hepatic immune response in vivo

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The role of hepatitis C virus (HCV) protein non-structural (NS) 5A in HCV-associated pathogenesis is still enigmatic. To investigate the in vivo role of NS5A for viral persistence and virus-associated pathogenesis a transgenic (Tg) mouse model was established. Mice with liver targeted NS5A transgene expression were generated using the albumin promoter. Alterations in the hepatic immune response were determined by western blot, infection by lymphocytic choriomeningitis virus (LCMV), and using transient NS3/4A Tg mice generated by hydrodynamic injection. Cytotoxic T lymphocyte (CTL) activity was investigated by the Cr-release assay. The stable NS5A Tg mice did not reveal signs of spontaneous liver disease. The intrahepatic immunity was disrupted in the NS5A Tg mice as determined by clearance of LCMV infection or transiently NS3/4A Tg hepatocytes in vivo. This impaired immunity was explained by a reduced induction of interferon β, 2'-5'OAS and PKR after LCMV infection and an impairment of the CTL mediated elimination of NS3-expressing hepatocytes. In conclusion, these data indicate that in the present transgenic mouse model NS5A does not cause a spontaneous liver disease. However, we discovered that NS5A could impair both the innate and the adaptive immune response to promote chronic HCV infection.

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The main green tea polyphenol epigallocatechin-3-gallate counteracts semen-mediated enhancement of HIV-infection

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Peptide fragments, derived from prostatic acidic phosphatase (PAP), are secreted in large amounts into human semen and form amyloid fibrils. These fibrillar structures, termed semen-derived enhancer of virus infection (SEVI), capture HIV virions and direct them to target cells. Thus, SEVI appears to be an important infectivity factor of HIV during sexual transmission. Here, we were able to demonstrate that epigallocatechin-3-gallate (EGCG), the major active constituent of geen tea, targets SEVI for degradation. Furthermore, it was shown that EGCG inhibits SEVI activity and abrogates semen-mediated enhancement of HIV-1 infection in absence of cellular toxicity. Therefore, EGCG appears to be a promising supplement of antiretroviral microbicides to reduce sexual transmission of HIV-1.

The nuclear pore component Nup358 promotes transportin-dependent nuclear import

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Nup358 (also known as RanBP2), a component of the cytoplasmic filaments of the nuclear pore complex, has been implicated in various nucleocytoplasmic transport pathways. Here, we identified Nup358 as an important factor for transportin-mediated nuclear import. Depletion of Nup358 resulted in a strong inhibition of nuclear import of the human immunodeficiency virus type 1 (HIV-1) Rev protein. HIV-1 Rev is an RNA-binding protein that is required for CRM1 (also known as exportin 1)-dependent nuclear export of unspliced or partially spliced viral RNA. We showed that transportin is the major nuclear import receptor for HIV-1 Rev in HeLa cells. Overexpression of transportin strongly promoted nuclear import of HIV-1 Rev in Nup358-depleted cells, indicating that the import receptor becomes rate-limiting under these conditions. Importantly, the import rate of other transportin-dependent proteins was also significantly reduced in Nup358-depleted cells. Our data therefore suggest a general role for Nup358 in transportin-mediated nuclear import.

Viral determinants for AIDS-progression in pediatric HIV-1 subtype B and C infections

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The course of pediatric AIDS progression is characterized by high viral titers accompanied by increased levels of immune activation and immune cell dysfunction. However, the viral determinants which are responsible for these effects are largely unknown. Accumulating data from in vitro experiments demonstrated that the HIV-1 Nef and Tat proteins lead to hyperactivation of virally infected T-cells. Furthermore, Nef was reported to cause B-cell dysfunction and hence hypergamma- immunoglobulinemia. The goal of our project is to assess the relevance of this in vitro findings in two cohorts of pediatric HIV-1 subtype B and C infections. In close collaboration with the UKE and the NICD we assembled blood samples and viral isolates from >50 HIV-1 subtype B and C infected children. The course of disease progression and clinical parameters are available for the majority of the patients enrolled in our study. HIV-1 nef and tat genes from all patient viral isolates will be amplified and analyzed for their capability to hyperactivate t-cells and to induce cytokines to stimulate uninfected bystander cells. Additionally, we will analyze multiple parameters which have been previously suggested to be important for disease progression. The data from the functional analysis of pediatric Nef and Tat proteins will be correlated to various clinical parameters from the patients, to elucidate viral determinants which are important for AIDS-progression.

Program Area "Cellular Dysregulation"

Head: Dr. Carol Stocking

Introduction

The cell has developed an elaborate arsenal to protect itself from dysregulation precipitated by either exogenous or endogenous stimuli. However, in the course of viral infections or during tumorigenesis, pathological mechanisms may be activated that bypass or inactivate these protective mechanisms, leading to the reprogramming of cellular pathways for the needs of viral replication or for successful tumor evolution. To understand these and other pathologic conditions, it is necessary to study in detail the basic mechanisms regulating cell growth and differentiation, as well as the safeguard pathways that ensure their proper function. The „Cellular Dysregulation Program" has several core themes that investigate both the normal regulatory controls and the viral or oncogenic mechanisms that disrupt these controls. During the last year, important advances have been made in each of these core themes, which are briefly outlined below. These results have not only revealed novel genetic pathways that are deregulated during virus infections or oncogenic transformation, but also provide new evidence that implicate human viruses in carcinogenesis. Furthermore, important findings have led to the identification of key cellular proteins initiating protective programs such as senescence or governing genomic stability, but also in regulating stem cell renewal and homing.

Research Projects

I. Targeted genes and pathways of oncogenic transformation

Met is an important target in SV40-induced mammary tumorigenesis

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We generated and characterized a transgenic mouse model (WAP-T mice) suitable for molecular analysis of initiation and progression of mammary adenocarcinoma. To identify genetic alterations related to tumorigenesis and tumor progression, we performed aCGH and microarray gene expression analyses of 65 tumor samples. The most prominent genetic alteration is an amplification of the *Met* (hepatocyte growth factor receptor) locus on chromosome 6 in undifferentiated compared to differentiated tumors, accompanied by a marked expression of Met. *Met* amplicons were not detected in spontaneous mammary tumors. In human breast cancer, strong expression of Met is observed in 20-30% of invasive ductal breast tumor specimens. Expression profiling and qPCR analysis revealed that in reference to the mammary gland of a BALB/c mouse matched in age and time of parturition to the tumor bearing animals, the Met gene is stronger expressed in all tumors; however significant variability between tumors was observed. In nearly all undifferentiated tumors a 10-20 fold higher expression of Met was detected. In order to determine the *Met* expression associated gene signature (Met signature) samples exhibiting a high *Met* expression level (Met^{high}) were compared with the group of samples showing low *Met* expression (Met^{low}). As the Met signature genes might have a prognostic and therapeutic significance, this gene list was used for cross-species analysis. Five human breast cancer expression datasets from the ArrayExpress and GEO repositories were selected for this analysis. Gene probe sets were matched between mouse and human gene expression microarray platforms, and after normalization clustered with the Met signature genes. Two clinical parameters, estrogen receptor status and survival of patients, were included in this statistical analysis, and the results were compared with recently published data. We demonstrated an association of Met^{high} with poor prognosis and observed that high *Met* expression itself and expression of a small subset of co-regulated genes is significantly associated with a negative ER status. This result let us to assume that Met^{high} tumors growing in WAP-T mice are related to human basal, triple-negative (ER-, PR-, ERBB2-) mammary carcinomas. The latter group of human cancer is also associated with loss or inactivation of p53, qualifying WAP-T tumors and cell lines derived from them as a model for triple-negative breast cancer.

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Transformation of human glial cells with adenovirus oncogenes

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Recently adenovirus DNA has been found in brain tumors providing a hint for a possible involvement of this virus in the tumorigenesis of neural tissues. To investigate, if the presence

of adenovirus in these cancers might be in correlation with the initial tumor formation, transformation experiments with primary glial cells derived from human adult retina have been performed. For this purpose the primary cells have been transduced with lentiviral vectors containing the Ad5 oncogenes E1A, E1B-55K, E4orf3 and E4orf6. Transduction with these constructs resulted in dense foci, which could be expanded into permanent cell lines. These displayed multiple additional properties commonly associated with a high grade of oncogenic transformation including morphological alterations, markedly enhanced growth rates and growth to much higher saturation densities. The transformation of primary human cells with adenoviral genes is known as a rare event, to date only described for embryonic cells. The formation of dense foci in glial cells provides the first example of adenoviral transformation in primary adult human cells. Moreover it supports the notion about a possible contribution of human adenoviruses for human malignancy, especially for neuroepithelial tumors.

Identification of novel cellular adenovirus type 5 E4orf6 interacting proteins

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The protein product from early region 4 (E4) open reading frame 6 (E4orf6) of human adenovirus type 5 is required for productive infection of human cells and for complete transformation of primary mammalian cells in cooperation with the E1A and E1B oncoproteins. The latter activity maps to multiple segments including the carboxy-terminal region, which has been implicated in nuclear retention of the viral protein. A peptide of this domain displays the characteristics of an arginine-faced amphipathic a-helix and overlaps with one of the two putative nuclear localization sequences. To gain further insight into the role of the E4orf6 oncodomain we have used the yeast two-hybrid system to isolate human cDNAs encoding proteins able to bind to the carboxy-terminal 91 amino acids. Two clones, designated E4-AP2 and E4-AP4 show substantial homology to several different cellular proteins known to be involved in nucleo-cytoplasmic trafficking and transcriptional regulation, respectively. The interaction between E4orf6 and E4-AP2/4 was verified by in vitro binding assays and by immunoprecipitation-immunoblot experiments with an epitope-tagged protein from transfected cells. We speculate that the interaction of E4orf6 with these factors may modify viral and cellular mRNA transport processes and transcription. Thus, E4-AP2 and E4-AP4 may represent at least two of the cellular targets that are modulated by E4orf6 in the neoplastic transformation of mammalian cells.

Adenovirus type 5 early region 1 B 55-kDa oncoprotein is modified by different isoforms of the small ubiquitin-related modifier (SUMO)

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We and others have previously shown that adenovirus type 5 (Ad5) E1B-55K is a substrate of the SUMO conjugation system in intact cells as well as *in vitro*. Posttranslational modification of E1B-55K by SUMO1 at lysine 104 (K104R) regulates the subcellular distribution of the

viral protein in plasmid transfected and productively infected cells. Furthermore, this modification is required to completely transform primary rat cells in cooperation with Ad5 E1A and to repress p53 tumor suppressor functions. Here, we report that E1B-55K can be modified by SUMO isoforms 2 and 3 (SUMO2, SUMO3) at K104. As predicted, the molecular characteristics of SUMO2 and SUMO3 give rise to additive SUMOylation of E1B-55K generating multiple modified forms of the viral protein. We also demonstrate that E1B-55K contains two amino acid patches, which closely resemble the consensus of SUMO-interaction motifs (SIMs). This motif, V/I-X-V/I-V/I mediates non-covalent interactions with SUMO or SUMOylated proteins, and exists in nearly all proteins known to be involved in SUMO-dependent processes, such as most notably PML. Using site-directed mutagenesis we show that these motifs regulate the cellular distribution of the Ad protein and are required for efficient repression of p53-mediated transactivation in transient reporter gene assays. Ongoing studies are directed towards elucidating the role of the SIMs in regulating protein interactions of 55K with viral and cellular factor as well as investigating their importance for E1B-55K functions in productive infection and cell transformation.

Transformation by Merkel Cell Polyomavirus (MCPyV)

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Merkel cell carcinoma (MCC) is a highly aggressive tumor of the skin which predominantly afflicts elderly and immunocompromised patients. The recently discovered Merkel cell polyomavirus (MCPyV) is believed to play a causative role in the pathogenesis of MCC, as clonally integrated MCPyV genomes are detected in ~80% of MCC cases. Interestingly, the large T-antigen (LT-Ag) gene of integrated MCPyV genomes unequivocally harbors mutations which selectively abrogate its ability to support viral replication while preserving its ability to target the cellular tumor supressor Rb. However, the transforming potential of MCPyV and especially full-length or truncated LT-Ag have not been investigated so far.

We have analyzed several existing and newly established MCC cell lines for the presence, integration pattern and copy number of MCPyV. Five MCPyV-positive lines were identified, all of which contained the LT-Ag truncating mutations characteristic for MCC tumors. From these lines, we subcloned four tumor-specific LT-Ag genes and generated an additional full length clone in which the truncating mutations were repaired. Upon ectopic expression, full-length as well as truncated LT-Ag efficiently induced focus formation and anchorage independent growth of REF52 as well as primary baby rat kidney (pBRK) cells. Interestingly, in three cases the truncated LT-Ag alleles displayed a higher transforming potential than the full length counterpart. This observation suggests that the truncating mutations may be the result of negative selection against viral replication as well as positive selection during the transformation process. The molecular basis for growth transformation elicited by shortened and full-length LT-Ag, as well as the contribution of constitutive LT-Ag expression to the transformed status of the various MCC cell lines are currently under investigation.

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Analysis of SPOC1's in vivo oncogenic potential

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The RNA expression level of SPOC1 is statistically correlated with a poorer prognosis and increased metastatic potential in a subset of ovarian cancer patients. Consistent with these findings it appears that SPOC1 protein expression levels are also increased in some other tumour tissues compared to non-malignant tissues arguing for a possible general role of enhanced SPOC1 expression in tumour development. Based on these observations and the fact that SPOC1 appears to be important for proper cell division we are currently evaluating SPOC1's oncogenic potential in vivo in immune compromised skid mice. In an effort to remain physiologically relevant and to recapitulate the wild-type situation we have generated several SPOC1 stable cell lines which show 3-10 fold over expression of the SPOC1 protein. Pilot experiments with one SPOC1 stable cell line indicate faster growing tumours in all mice in comparison to the parental control cell line. Based on these pilot experiments we are now planning a large scale statistically significant repeat of these in vivo tumour growth studies using several different SPOC1 stable cell lines. The results obtained from these experiments should help to clarify the oncogenic potential of SPOC1 over expressing cells as well as any metastatic relationship affiliated with SPOC1 over expression.

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The MADS transcription factor MEF2C is an important regulator of monopoiesis and the leukemic stem cell compartment in MLL-associated leukemia

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Acute myelogenous leukemia (AML) is driven by leukemic stem cells (LSCs) generated by mutations that confer (or maintain) self-renewal potential coupled to an aberrant differentiation program. Using retroviral mutagenesis, we have identified genes that generate LSCs in a mouse model for myeloproliferation induced by *Irf8* deficiency. Among the targeted genes, we identified *Mef2c*, encoding a *MCM1-agamous-deficiens-serum response factor* (MADS) transcription factor. Using a retroviral gene transduction – bone marrow transplantation model we have been able to demonstrate the importance of MEF2C in AML development in susceptible mice. This analysis has also shown the importance of MEF2C in lineage commitment to the monocytic lineage, which has been corroborated by analysis of conditional *Mef2c* knock-out mice. Mechanistic studies have shown that loss of functional Mef2c correlated with reduced levels of transcripts encoding c-Jun, but not PU.1, C/EBPalpha, or JunB transcription factors. Inhibiting Jun expression by short-interfering RNA impaired Mef2c-mediated inhibition of granulocyte development. Moreover, retroviral expression of c-Jun in BM cells promoted monocytic differentiation. The ability of Mef2c to modulate cell-

fate decisions between monocyte and granulocyte differentiation, coupled with its functional response to extracellular stimuli, demonstrate an important role in immunity and – consistent with findings of other myeloid transcription factors – a target of oncogenic lesions in AML.

Strikingly, several of the genes identified in our screen have been reported to be up-regulated in the mixed lineage leukemia (MLL) subgroup of leukemia marked by disruption of the *MLL* gene and with a poor prognosis. High MEF2C expression levels were confirmed in AML patient samples with *MLL* gene disruptions, prompting an investigation of the causal interplay. Using a conditional mouse strain, we demonstrated that Mef2c deficiency does not impair the establishment or maintenance of LSCs generated in vitro by MLL/ENL fusion proteins; however, its loss led to compromised homing and invasiveness of the tumor cells. Mef2c-dependent targets included several genes encoding matrix metalloproteinases and chemokine ligands and receptors, providing a mechanistic link to increased homing and motility. Thus, MEF2C up-regulation may be responsible for the aggressive nature of this leukemia subtype.

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Hidden force of p53: Homeostatic behavior of self-sustaining, hierarchically organized tissue cell populations

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Cell differentiation is the fundamental process of gradual commitment of a dividing stem/progenitor cell whose progeny creates a self-sustaining, hierarchically organized population of specialized cells. Inactivation or alteration of regulatory pathways due to mutations or epigenetic aberrations in stem/progenitor cells misbalance the homeostatic behavior of tissue cell populations and provoke tumorigenesis. As tumor growth is often accompanied by loss or modification of p53 protein function, we assumed that the transcriptional regulator p53 is involved in controlling proliferation and differentiation of stem/progenitor cells. Mouse F9 teratocarcinoma cells constitute a well studied model system for embryo-derived cancer cell differentiation. Under standard cell culture conditions the initiation of F9 cell differentiation requires supply of a potent inductor, retinoic acid, which activates conversion of rapidly proliferating F9 cells into fully differentiated extraembryonal parietal and visceral endodermal cells. Upon growth of F9 cells in a hollow fiber-based culture, CarboCell tubes, providing nearly physiological conditions (proper cell-cell contacts and concentration of secreted factors), no inductor is required for endodermal cell differentiation. Using biochemical assays for stem/progenitor cells we analyzed the standard and hollow fiber-based F9 cell cultures and show that a subpopulation of F9 cells with high activity of the stem cell marker aldehyde dehydrogenase ($ALDH^{bright}$ cells) comprises the F9 stem cell population. $ALDH^{bright}$ cells also contain high levels of functional p53. We show that p53 plays a role in controlling stemness, and also provide phenomenological evidence for a role of p53 in the regulation of asymmetric cell division, which is a hallmark of stemness. As loss of the tight control over

cell fate regulation in normal stem/progenitor cells such cells leads to their expansion via symmetric cell divisions, our data establish a link between the loss or alteration of p53 function and the transformation of normal stem/progenitor cells to cancer cells.

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II. Tumor suppressors and regulation of apoptosis, senescence and proliferation

Rb2/p130, the dominant pocket protein in cellular senescence

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The different pocket proteins are established as negative cell cycle regulators. With regard to the repressor functions of pocket proteins in cellular senescence, studies so far have mainly focused on pRb/p105. Here, we show that in a broad range of wild-type p53-expressing human tumor cells, and in human diploid fibroblasts, Rb2/p130 is the dominating pocket protein in replicative and in accelerated senescence. Senescent cells are arrested at the transition from late G1- to early S-phase, as indicated by the absence of S- and G2-phase cyclins A and B. Expression of cyclin A and entry into S-phase resumed after RNA interference-mediated knockdown of Rb2/p130. Activation of different upstream pathways by overexpression of either p21 or p16 converged on Rb2/p130 accumulation and induced senescence. In contrast, p53- or p21-negative cells treated with DNA-damaging agents failed to accumulate Rb2/p130 and to enter senescence. Our data suggest that Rb2/p130 is a member of the p53-p21 DNA damage signaling cascade, and represents the essential pocket protein family member needed for the induction of any type of senescence.

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Mutant p53 gain of function in mammary carcinogenesis

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Missense mutations and loss of the *wild-type* allele in the *TP53* gene frequently accompany tumor formation. The most prevalent missense mutations found in human breast cancer are at the positions R248 and R273. The *p53* mutant allele encodes a protein (mutp53), which has lost the transcriptional activity towards pro-apoptotic and growth arrest genes, but has retained the propensity to interact with nucleic acids and proteins. To study the influence of mutp53 on the development of SV40-induced mouse mammary carcinoma we developed bi-transgenic WAP-T (SV40 early region) x WAP-mutp53 mouse models with equivalent mutations (R245W, R270H). Mutp53 acts synergistically with SV40 T/t-antigens and enhances tumour incidence, growth and pulmonary metastasis.

To dissect the molecular mechanisms of mutp53 function we performed gene expression profiling of tumor and normal tissues. By bioinformatic evaluation of microarray data we

identified a small set of genes whose expression strongly correlates with mutp53. We focused on *Apobec3, a* gene known to be involved in the intrinsic anti-viral response by RNA-editing. Tumors and involuted glands of WAP-T-mutp53 (R245W) bi-transgenic animals revealed a higher Apobec3 expression than the respective tissues of WAP-T mono-transgenic mice. These preliminary results indicate a role of Apobec3 in tumor progression and aggressiveness. We are currently investigating the mode of Apobec3 regulation by mutp53 and its possible role in mutp53 gain of function. Specifically, we test the hypothesis that mutp53 may influence miRNA expression and in this way provide an additional level of regulation.

Another so far unknown function of mutp53^{R270H} became apparent, when we tried to establish cell-lines from WAP-T monotransgenic and WAP-T x WAP-mutp53 mammary carcinomas. Surprisingly, until now, only tumor-cells bearing mut-p53^{R270H} were able to survive under cell-culture conditions and retain their epithelial phenotype.

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The RUNX1 transcription factor: A gatekeeper in acute leukaemia

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RUNX1 is one of the most frequently mutated genes in human acute leukemia and hematopoietic disorders. It is directly targeted by chromosomal translocations in 15 to 20% of both acute myelogenous leukemia (AML) and pediatric B-cell acute lymphoblastic leukemia (ALL) patients. In addition to translocations, inactivating or DNA-binding mutations in the *RUNX1* gene have been identified in the minimally differentiated M0 AMLs and in myelodysplastic syndromes with a propensity to develop AML. Unraveling the reported tumor suppressor and oncogenic functions of the wild-type, mutated, or fusion proteins is essential to develop novel therapeutic approaches. Over the last few years, we have established mouse models for the commonly found mutated or fusion proteins using a retroviral-vector transduction protocol coupled with bone marrow transplantation. The coexpression of GFP is this model has allowed the evaluation of changes in the hematopoietic system before the onset of an overt leukemia. The evaluation of these mice have lead to the following conclusions: 1) intrinsic functions of the fusion protein or mutated protein play a determining role in the phenotype (myeloid vs. lymphoid) of the ensuing leukemia; 2) whereas repression of direct target genes of RUNX1 is a common functional denominator of RUNX1 fusion proteins, DNA-binding-independent functions of RUNX1 play an important role in leukemogenesis; 3) RUNX1 gene alterations alone are insufficient to induce a leukemia, but probably act as gatekeeper, permitting the outgrowth of a preleukemic clone, which is the target of secondary events. Our recent work has shown that DNA-binding mutations of RUNX1 synergize with activated FLT3, a receptor tyrosine kinase, to induce an AML in mice. Interestingly, prelimi-

nary results suggest that loss of both *Runx1* alleles is necessary for disease induction, confirming a tumor suppressor role of Runx1 in the presence of activating FLT3 mutations. Furthermore, a role for the RUNX genes in cancer fail-safe processes has been confirmed by demonstrating RUNX1 mediated senescence-like growth arrest in human primary fibroblasts. Senescence induction required p16 (ink4a) and a correlation was noted between induction of p53 (in the absense of p14), reactive oxygen species and phospho-p38, whereas p38(MAPK) inhibition markedly rescued cell growth. These findings indicate a role for replication-independent pathways in RUNX senescence.

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In vitro senescence of mesenchymal stem cells

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Mesenchymal stem cells (MSCs) are of particular interest because they are being tested in cell and gene therapies for a number of human diseases. MSCs represent a rare population in tissues. Therefore, it is essential to grow MSCs in vitro before putting them into therapeutic use. This is compromised by senescence, limiting the proliferative capacity of MSCs. We analyzed the in vitro senescence of rat MSCs, because this animal is a widespread model for preclinical cell therapy studies. After initial expansion, MSCs showed an increased growth doubling time, lost telomerase activity, and expressed senescence-associated beta-galactosidase. Senescence was accompanied by down-regulation of several genes involved in stem cell self-renewal. Of interest, several genes involved in DNA repair also were significantly down-regulated. Entry into senescence occurred with characteristic changes in retinoblastoma (RB) expression patterns. Rb1 and p107 gene expression decreased during in vitro cultivation. In contrast, pRb2/p130 became the prominent RB protein. This suggests that RB2/P130 could be a marker of senescence or that it even plays a role in triggering the process in MSCs.

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Nestin modulates glucocorticoid receptor function

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Nestin is the characteristic intermediate filament (IF) protein of rapidly proliferating progenitor cells and regenerating tissue. Nestin copolymerizes with class III IF-proteins, mostly vimentin, into heteromeric filaments. Its expression is downregulated with differentiation. Here we show that a strong nestin expression in mouse embryo tissue coincides with a strong accumulation of the glucocorticoid receptor (GR), a key regulator of growth and dif-

ferentiation in embryonic development. Microscopic studies on cultured cells show an association of GR with IFs composed of vimentin and nestin. Cells lacking nestin, but expressing vimentin, or cells expressing vimentin, but lacking nestin accumulate GR in the nucleus. Completing these networks with an exogenous nestin, respectively an exogenous vimentin restores cytoplasmic anchoring of GR to the IF system. Thus, heteromeric filaments provide the basis for anchoring of GR. The reaction pattern with phospho-GR specific antibodies and the presence of the chaperone HSC70 suggest that specifically the unliganded receptor is anchored to the IF system. Ligand addition releases GR from IFs and shifts the receptor into the nucleus. Suppression of nestin by specific shRNA abolishes anchoring of GR, induces its accumulation in the nucleus and provokes an irreversible G1/S cell cycle arrest. Suppression of GR prior to that of nestin prevents entry into the arrest. The data give evidence that nestin/vimentin specific anchoring modulates growth suppression by GR. We hypothesize that expression of nestin is a major determinant in suppression of anti-proliferative activity of GR in undifferentiated tissue and facilitates activation of this growth control in a precise tissue and differentiation dependent manner.

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High expression of the immature laminin receptor protein predicts a good prognosis in B-cell chronic lymphocytic leukaemia

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The immature laminin receptor (iLR) is a tumor-associated antigen expressed on the surface of various tumor entities including hematological malignancies. In the current study, we analyzed the expression of iLR on malignant B cells of 134 unselected patient samples with chronic lymphocytic leukemia (CLL) and hypothesized that iLR expression would have prognostic significance. ILR expression determined by flow cytometry (cut-off value 30%) was strongly correlated with no requirement for treatment (P<.0001) and mutated immunoglobulin VH (IgVH) status (P<.0001). ILR is not expressed on normal B-cells and was not upregulated upon stimulation with CD40L, CpG oligonucleotides or B cell receptor ligation. Microarray analysis of iLR-negative and iLR expressing B-CLL cells showed differential expression patterns of several gene products. Patients with high iLR-expression had a significantly longer time to first treatment (p<0.0001) and overall survival (p<.01). Combination of CD38 (>20%), ZAP70 (>30%), and iLR (<30%) by flow cytometry can be used to construct a prognostic model identifying patients with a median progression free survival of 80 months, if no adverse marker is present. The constant and tumor selective surface expression and the strong correlation with other established prognostic markers make iLR a new valuable marker for assessment of risk profile in CLL.

The human RNA polymerase II-associated factor 1 (hPaf1): A new regulator of cell-cycle progression

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The human PAF (hPAF) complex is part of the RNA polymerase II transcription apparatus and regulates multiple steps in gene expression. Further, the yeast homolog of hPaf1 has a role in regulating the expression of a subset of genes involved in the cell-cycle. We therefore investigated the role of hPaf1 during progression of the cell-cycle. METHODOLOGY/FINDINGS: Herein, we report that the expression of hPaf1, a subunit of the hPAF complex, increases with cell-cycle progression and is regulated in a cell-cycle dependant manner. hPaf1 specifically regulates a subclass of genes directly implicated in cell-cycle progression during G1/S, S/G2, and G2/M. In prophase, hPaf1 aligns in filament-like structures, whereas in metaphase it is present within the pole forming a crown-like structure, surrounding the centrosomes. Moreover, hPaf1 is degraded during the metaphase to anaphase transition. In the nucleus, hPaf1 regulates the expression of cyclins A1, A2, D1, E1, B1, and Cdk1. In addition, expression of hPaf1 delays DNA replication but favors the G2/M transition, in part through microtubule assembly and mitotic spindle formation. CONCLUSION/SIGNIFICANCE: Our results identify hPaf1 and the hPAF complex as key regulators of cell-cycle progression. Mutation or loss of stoichiometry of at least one of the members may potentially lead to cancer development.

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Identification of genetic mutations that cooperate with constitutively active FLT3 in the induction of acute lymphoblastic leukemia (ALL)

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Acute lymphoblastic leukemia (ALL) is the most common form of childhood malignancy and is a heterogeneous disease with subtypes that differ markedly in their cellular and molecular characteristics, as well as their response to therapy and subsequent risk of relapse. The FMS-like receptor tyrosine kinase FLT3 contributes to normal differentiation, proliferation and apoptosis of primitive hematopoietic cells in both early myeloid and lymphoid lineages. Mutations constitutively activating the FLT3 receptor are commonly found in acute leukemias of both lineages, making FLT3 one of the most frequently mutated genes in hematological malignancies. The availability of FLT3 inhibitors provides an attractive therapeutic strategy for patients harboring these mutations. In ALL, FLT3 mutations are associated with either MLL or hyperdiploid subtypes, raising the question if specific genetic events synergize with FLT3 mutations in ALL induction and if inhibiting FLT3 activation alone will be effective

in leukemia treatment. To better understand the role of FLT3 activating mutations in ALL, we have established an allograft mouse model, in which bone marrow (BM) progenitors were transduced with a retroviral vector expressing FLT3 with an internal tandem duplication (FLT3-ITD), followed by transplantation into syngenic recipients. Mice receiving murine FLT3-ITD bone marrow succumbed to an aggressive leukemia with a mean latency of 45 days. Based on surface marker analysis and immunoglubulin (Ig) gene rearrangements, these tumors could be classified as pre-B-cell tumors. The disease is transplantable and clonal (based on retroviral integration sites). Strikingly, the FLT3 inhibitor PKC412, as well inhibitors of the PI3K and MAPK pathways or dominant-negative forms of STAT5, profoundly inhibited the proliferation and survival of leukemic cells *in vitro*. Each leukemia contained up to 6 to 8 independent retroviral integrations sites, a much higher number than would be expected given the circa 15-20% infection frequencies of BM cells before transplantation. The high number of integrations could reflect two events: 1) preferential infection of a subset of cells more permissive to infection; and/or 2) the preferential outgrowth of these infected cells due to the increased likelihood that cooperating genes were activated through the integration site. To determine if integration of the retroviral vector near putative oncogenes or tumor suppressors contributed to the induction of leukemia, retroviral integration sites were analyzed. Consistent with this hypothesis, integrations were found near genes that have been identified as “common integration sites” (CIS) or loci that are targeted in independent tumors – an event highly unlikely to occur merely by chance.

Based on the function of gene products deregulated by the retroviral integration, we predict that signals downstream of the preB-cell receptor are disrupted in this leukemia, which may be responsible for the block in differentiation. We are currently determining if these type of mutations also synergize with activating JAK2 mutations, recently identified in 10% of high-risk ALL patients.

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III. Genomic instability and DNA repair mechanisms

Induction of genomic instability as a basis for virus-mediated oncogenesis

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Adenovirus-mediated oncogenic transformation is a multistep process that involves a complex interplay between viral and cellular proteins, insertional mutagenesis by viral DNA integration and epigenetic changes as well as the induction of mutations in cellular genes. There is good reason to believe that accumulation of mutations is probably triggered by the cooperation of various factors, including the unscheduled induction of cellular DNA synthesis by adenovirus (Ad) E1A as well as the modulation of p53 and PML NBs by Ad E1B and E4 oncoproteins. The later possibility is intriguing because other viral oncoproteins similarly implicated in the induction of mutations, such as SV40 Tag and HCMV IE1/IE2 also modulate the function of p53 as well as the integrity of PML NBs. Perhaps more significant, the reported inhibition of DNA double-strand break repair by Ad E4 through binding to key regulators of the DNA damage response (DNA-PKcs, Mre11/Rad50/NBS1, DNA Ligase IV, and PP2A) could potentially contribute to the induction of mutations and genetic instability, thus forming the basis for virus-mediated oncogenesis. To test this model we have established a novel

transformation system with primary mammalian cells and lentiviral vectors, which will allow us to measure virus-induced mutagenesis and the induction of genomic instability at the molecular level.

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Adenovirus 12 E4orf6 inhibits ATR activation by promoting TOPBP1 degradation

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Activation of the cellular response to DNA damage is detrimental to adenovirus (Ad) infection. ATM and ATR are the two principal kinases that control this response; therefore, Ad has evolved a number of different strategies to inhibit ATM- and ATR-dependent signaling during infection. Recent work suggests that the Ad5 E4orf3 protein prevents ATR activation through its ability to mislocalize the MRE11-RAD50-NBS1 (MRN) complex. Here, we provide evidence to indicate that Ad12 has evolved a different strategy from Ad5 to inhibit ATR. We show that Ad12 targets the ATR activator protein, TOPBP1, for ubiquitin-dependent, proteasome-mediated degradation. RNA interference experiments demonstrated that Ad12 requires a CUL2/Elongin C/RBX1-containing ubiquitin ligase to promote TOPBP1 degradation. We also found that Ad12 utilizes this complex to degrade p53 during infection, in contrast to Ad5, which requires a CUL5-containing ubiquitin ligase. Furthermore, we determined that TOPBP1 degradation by Ad12 does not require E1B-55K, and is solely dependent on the expression of E4orf6. We propose that Ad12 E4orf6 has two principal activities: to recruit the CUL2-based ubiquitin ligase, and to act as its substrate receptor, by binding directly to TOPBP1 and promoting its polyubiquitylation. Finally, in support of the notion that Ad12 E4orf6 specifically inhibits CHK1 activation during infection by targeting TOPBP1 for degradation, we demonstrate that Ad12 E4orf6 expression can similarly attenuate the ATR-dependent phosphorylation of CHK1 in response to replication stress. Taken together, these data provide novel insights into how Ad modulates ATR signaling pathways during infection

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Modulation of chromatin associated proteins by SPOC1 in response to DNA damage

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In eukaryotic cells and viruses, type and extend of DNA damage induced by endogenous and exogenous factors as well as the selection of pathways which lead to recognition and repair of specific DNA lesions strongly depend on chromatin structure and its modulation. As we have recently discovered that **SPOC1** (**S**urvival time associated **P**HD finger in **O**varian **C**ancer **1**) is a protein dynamically associated with and structurally modifying chromatin structure in a cell cycle dependent manner we began to investigate systematically whether deregula-

tion of SPOC1 expression effects expression levels, subcellular distribution, and posttranslational modification of chromatin associated proteins with known functions in DNA repair and chromatin structure. Experimental up- and down-regulation of SPOC1 expression in various cell lines provided preliminary evidences for a major impact of SPOC1 expression levels on several chromatin and DNA repair associated proteins upon DNA damage induction. Therefore, modulation of SPOC1 expression is likely to affect different DNA repair pathways, oncogenic processes and viral infections.

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The intra-S-phase checkpoint: Coordinator of repair and replication activity

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Our investigations demonstrated that γ-irradiated primate cells, damaged at the G1/S-transition, activate the ATM/Chk2-mediated intra-S-checkpoint, which transiently delays S-phase progression and onset of replication for two hrs. In addition, four hrs after γ-irradiation activation of the ATR/Chk1-mediated signaling cascade was noticed an event that correlated with active DNA-replication. Accordingly, data indicate that in γ-irradiated G1/S cells ATM and ATR are activated and inactivated consecutively by two different DNA damage-inducing events. Thus, the presence of γH2AX-foci as an indicator for DNA-double-strand breaks (DSBs) was determined at specific time points post G1/S-irradiation (hpi). Measuring the kinetics of γH2AX-foci formation using a confocal cell imaging system revealed two discrete peaks, which appeared before and during replication. Data suggest that the first γH2AX-peak reflects radiation-induced DSBs, whereas the second one appears to be replication-induced. The disappearance of γH2AX-foci immediately after the DNA duplicating process indicates that DSBs have been repaired when cells progress into G2. Since DSBs can be repaired by non-homologous end joining (NHEJ) as well as homologous recombination-directed repair (HDR), the formation of Rad51-foci, an indicator for HDR, was investigated as a function of time. Results demonstrated that Rad51-foci were only detectable in replicating cells but not during the two hr ATM-mediated attenuation period. The finding suggests that the primary, radiation-induced DSBs are repaired by NHEJ, whereas the secondary DSBs, generated by simultaneous base excision repair (BER) and replication activity, are repaired by HDR. Moreover, inhibition of replication abrogated Rad51- but not γH2AX-foci formation, suggesting that the replication process is essential to promote homologous recombination-directed repair of DSBs in S-phase cells. Taken together, data demonstrate that γ-irradiated G1/S cells activate the intra-S checkpoint involving the ATM as well as the ATR signaling cascades, thereby promoting repair of primary and secondary DSBs by NHEJ and HDR, respectively. Accordingly, the findings provide insight into the ATM/ATR-mediated interplay between DSBs repair and the DNA-replication process in damaged S-phase cells.

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Role of the p53 gene family in the intra-S-phase checkpoint

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Our investigations demonstrated that the UV-induced ATR/Chk1-signaling pathway causes transient delays in S-phase progression and reversible inhibition of DNA replication for six hrs. The key players of this pathway, which allow extended S-phase attenuation and inhibition of origin-dependent replication initiation, are transcriptional active wtΔp53 and transcriptional impaired wtp53, respectively. The transcriptional activity of Δp53 leads to p21-mediated down regulation of cyclin A-Cdk2 activity, an activity that is required for S-phase progression and processive DNA synthesis performed by polymerases δ and ε. Down regulation of cyclin A-Cdk2 activity is a prerequisite for facilitating interaction of wtp53 with hypophosphorylated, origin-competent DNA polymerase α-primase (Pol α), an event that prevents origin loading of Pol α and consequently initiation of DNA replication. Therefore, the Δp53- and the p53-dependent sequential branches of the ATR-mediated intra-S checkpoint are essential to slow down S-phase progression and to prevent origin firing. Apart from replication inhibition, the intra-S checkpoint-mediated S-phase attenuation provides another critical function: error-free repair of damaged DNA before DNA replication is initiated. The assumption is based on the fact that inactivation of Δp53 or its gene product p21 abrogates extended S-phase attenuation, resulting in accelerated, faulty repair of mutagenic (6-4)PP lesions giving rise to secondary DNA damage. Moreover, UV-irradiated cells proficient in the ATR-Δp53-p21-cyclin A-Cdk2 pathway but deficient in functional wtp53 initiate origin-dependent DNA replication and acquire DNA double strand breaks in the absence of processive DNA replication. Although secondary DNA damages are repaired in the subsequent G2-phase, these cells may have already missed their best opportunity to perform error-free repair before replication is initiated.

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Elucidation of the possible role of SPOC1 cell cycle regulation and DNA replication

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Survival time associated **P**HD protein in **O**varian **C**ancer **1** (SPOC1) is statistically correlated with a poorer prognosis in a subset of ovarian cancers. In an effort to understand this relationship we have undertaken a detailed analysis of the SPOC1protein. We have recently been able to show that SPOC1 is a tightly regulated protein with differential expression during the cell cycle. Based on the observations that SPOC1 protein expression is highest during mid-late S-phase and during mitosis we postulated that SPOC1 may have multiple cell cycle related functions. Supporting this possibility are the findings that siRNA mediated knockdown of SPOC1 results in various mitotic defects, whereas it’s induced over expression in SPOC1 stable cell lines causes a G2 cell cycle delay implicating replicative stress. A closer analysis of mitosis revealed that SPOC1 has a biphasic affiliation with chromatin during mitotic progression, specifically during early-middle prophase and then later and during mid-late anaphase and telophase. Interestingly, this dynamic regulation is synonomous with the chromosome condensation events of mitosis. By the use of MNase

assays we were further able to demonstrate that SPOC1 protein concentration is proportionally related to the degree chromatin condensation. Together, these findings implicate an important biological role of the SPOC1 protein in cell division. To further evaluate the role of SPOC1 in cellular replication we are currently employing various experimental approaches including synchronization, EDU labelling, live cell microscopy and immunoprecipitation. Accordingly, we hope to be able to identify the exact cellular function of SPOC1 during replication. We currently speculate that SPOC1 may play a role in the recruitment of different replication proteins to the chromatin. Elucidation of the exact cellular functions of SPOC1 will prove to be useful in understanding the underlying relationship of SPOC1 and tumorigenesis.

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Down-regulation of hypusine biosynthesis in plasmodium by inhibition of S-adenosyl-methionine-decarboxylase

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An important issue facing global health today is the need for new, effective and affordable drugs against malaria in particular in resource-poor countries. Moreover, the currently available anti-malarials are limited by factors ranging from parasite resistance to safety, compliance and cost. Innovations in medicinal chemistry are presently lacking.

Depletion of polyamines in the intraerythrocytic phase of *P. falciparum* is a promising strategy for the development of new antimalarials since intracellular levels of putrescine, spermidine and spermine are increased during cell proliferation. S-adenosyl-methionine-decarboxylase (AdoMETDC) is a key enzyme in the biosynthesis of spermidine. The AdoMETDC inhibitor CGP 48664A, known as SAM486A, inhibited the separately expressed plasmodial AdoMETDC domain with a K_i of 3 µM resulting in depletion of spermidine. Spermidine is an important precursor in the biosynthesis of hypusine. This prompted us to investigate a downstream effect on hypusine biosynthesis after inhibition of AdoMETDC. Extracts from *P. falciparum in vitro* cultures which were treated with 10 µM SAM 486A showed suppression of eukaryotic initiation factor 5A (eIF-5A) in comparison to the untreated control in two-dimensional gel electrophoresis. Depletion of eIF-5A was also observed in Western Blot analysis with crude protein extracts from the parasite after treatment with 10 µM SAM486A.

A determination of the intracellular polyamine levels revealed an approximately 27% reduction of spemidine and a 75% decrease of spermine while putrescine levels increased to 36%. These data suggested that inhibition of AdoMetDc provides a novel strategy for eIF-5A suppression and the design of new anti-malarials.

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IV. Epigenetic controls and chromatin organization

Mutant and wild-type p53 as modulators of global chromatin organization

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Missense mutations in the *TP53* gene are frequent genetic alterations in human tumor tissue and cell lines. In contrast to its wild-type counterpart, the mutant p53 (mutp53) protein is long-lived and has lost the transcriptional activity towards pro-apoptotic and growth arrest genes, but retained the propensity for being targeted to chromatin. Expression of mutp53 is advantageous for tumor cells, however the molecular mechanism of mutp53 action is still not known. We used tumor cell lines expressing endogenous and inducible mutp53 proteins as models to study the role of mutp53 in transcriptional regulation. Mutp53 has lost sequence specificity in DNA binding activity but retained the property to recognize DNA secondary structures, and based on our ChIP-Seq data is prone to interact preferentially with repetitive sequences possessing conformational flexibility. Therefore we assumed that mutp53 operates on the level of global chromatin organization rather than on modulating the expression of individual genes. In support, we found that differentially regulated genes frequently map to the same chromosomal locations or even are organized as physically-linked gene clusters. This implies that mutp53 might be a factor involved in regulating the recruitment of gene promoters to immobile transcription factories containing active RNA polymerases. To test this hypothesis, physical DNA contacts in several genomic loci encompassing co-regulated gene clusters were profiled using the chromosome conformation capture (3C) method. Preliminary results strongly support the proposed function of mutp53 in modulating the gene expression program at a higher level.

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SPOC1: A modulator of chromatin epigenetics and structure

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The SPOC1 protein contains in its C-terminus a sequence with strong homology to plant homeodomains (PHD) which are frequently found in proteins associated with chromatin and often bind to histones modified posttranslationally at specific sites We tested whether this applies also to the PHD in the context of the SPOC1 protein sequence. We found that SPOC1 is primarily affiliated with chromatin as determined by differential fractionation assays. Moreover, we have been able to show via EMSA, pull-down assays as well as by peptide chips that SPOC1 can in fact bind DNA, histones and nucleosomes which are all constituents of chromatin. Interestingly, transient over expression of the SPOC1 protein results in global chromosome condensation, a phenotype that is eliminated by the deletion of its

C-terminal PHD domain. In stark contrast to these observations, SPOC1 siRNA mediated knockdown results in the decondensation of chromatin. Together these findings implicate a role of SPOC1 in the regulation of chromatin structure. To better understand the mechanisms behind SPOC1 induced chromatin structural changes we are currently surveying the epigenetic repertoire in wild-type and SPOC1 over expressing cell lines. Elucidation of the differential epigenetics affording such chromatin structural changes will provide important clues for the identification of the affiliated chromatin remodelling proteins. Answers to these questions should provide significant insights into how SPOC1 can induce chromatin structural changes as well as its biological relationship with other important chromatin modulating proteins. We are confident that SPOC1 has also an impact of chromatin structure of viral DNA and will analyse this in the near future.

SPOC1: A novel PHD-containing protein modulating chromatin structure and mitotic chromosome condensation.

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In this study, we characterize the molecular and functional features of a novel protein called SPOC1. SPOC1 RNA expression was previously reported to be highest in highly proliferating tissues and increased in a subset of ovarian carcinoma patients, which statistically correlated with poor prognosis and residual disease. These observations implied that SPOC1 might play a role in cellular proliferation and oncogenesis. Here we show that the endogenous SPOC1 protein is labile, primarily chromatin associated and its expression as well as localization are regulated throughout the cell cycle. SPOC1 is dynamically regulated during mitosis with increased expression levels and biphasic localization to mitotic chromosomes indicating a functional role of SPOC1 in mitotic processes. Consistent with this postulate, SPOC1 siRNA knockdown experiments resulted in defects in mitotic chromosome condensation, alignment and aberrant sister chromatid segregation. Finally, we have been able to show, using micrococcal nuclease (MNase) chromatin-digestion assays that SPOC1 expression levels proportionally influence the degree of chromatin compaction. Collectively, our findings show that SPOC1 modulates chromatin structure and that tight regulation of its expression levels and subcellular localization during mitosis are crucial for proper chromosome condensation and cell division.

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Identification of SPOC1 chromatin affiliated complexes

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SPOC1 is primarily a chromatin associated protein that shows dynamic regulation in its expression during the cell cycle. Several evidences lead us to believe that the SPOC1 protein is an integral component of chromatin remodelling complexes potentially with diverse cellular functions. Therefore in an effort to elucidate the proteomic network/s affiliated with SPOC1 we have started purifying SPOC1 chromatin associated complexes in synchronized and asynchronous cells using column chromatography, immunoprecipitation and will analyse them by mass spectrometry. In parallel we initiated a search for SPOC1 interaction partners by use of yeast 2-hybrid systems. These pilot experiments have resulted in the identification of several putative interaction partners. These preliminary data are consistent with our assumption that SPOC1 can modulate chromatin and chromosome structures by recruitment and displacement of known chromatin assiated protein and chromatin modifying enzymes. Using eukaryotic and prokaryotic purified SPOC1 full length protein and deletion fragments, we hope to be able to directly map the binding domains of the identified complex proteins. Through the combination of these approaches we are optimistic to identify the components of SPOC1 chromatin modifying complexes which will ultimately help to define the functional responsibilities of the SPOC1 protein. Preliminary results to date suggest that SPOC1 maybe a component of different chromatin affiliated complexes of diverse molecular weights and that its interactome likely involves important cell cycle regulating and chromatin modifying proteins.

Supported by the Deutsche Krebshilfe

Analysis of mechanisms of polyploidisation and chromosome translocations induced by enhanced SPOC1 protein expression

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The role of SPOC1 as a novel oncoprotein was first implied by the correlation of its RNA expression levels with survival time and metastatic potential in a subset of ovarian carcinomas. In line with these early indications that suggested miss regulation of SPOC1 expression levels may favour oncogenic transformation and/or metastasis, we now have direct evidence in two different cell types and multiple cell clones, that higher expression of SPOC1 severely compromises genomic integrity. Using this cell culture approach we have found that over expression of SPOC1 (2-10 fold) leads to both polyploidization and to chromosome translocations in a dosage dependent manner. These results demonstrate that altered SPOC1 expression levels directly leads to chromosome instability and changes in cellular ploidy, characteristic hallmarks of tumours. These findings not only support the postulate that SPOC1 may be a novel oncoprotein but also implicate that SPOC1 may be a valuable tumour

diagnostic and prognostic marker. In an effort to better understand the mechanism behind SPOC1 induced polyploidization and chromosome translocations we are currently performing a detailed dissection of the control mechanisms and checkpoints regulating replication and cell division. Preliminary findings thus far suggest that SPOC1 induced polyploidization and chromosome translocations occur via the prolonged chromatin affiliation of replication and repair specific proteins. Whether or not SPOC1 mediates the prolonged recruitment of these proteins to the chromatin through altered epigenetics or directly via protein-protein interactions still remains to be determined.

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Analysis of the role of SPOC1 in spermatogenesis

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Recently, we could show that SPOC1, a novel PHD-finger protein, is associated with chromatin and plays an important role in the condensation of chromatin and during mitosis. SPOC1 RNA and protein are highest expressed in the testis, whereas considerably lower amounts are observed in all other types of tissues tested so far. This observation prompted us to investigate in more detail the expression and the putative functions of the SPOC1 protein in the testis. Using different marker proteins, we could demonstrate that the SPOC1 protein is expressed in spermatogonia, which are the mitotic germ cells of the adult testis, but not in other types of germ cells (meiotic spermatocytes, spermatids). We could further demonstrate that in the mouse testis, SPOC1 is expressed both in undifferentiated (PLZF-positive) as well as in differentiating (Sohlh1-positive) spermatogonia. In a mouse model with inducible knockdown of SPOC1 we found that even partial reduction of SPOC1 results in a progressive loss of germ cells in the seminiferous tubules in the testes of these mice with time, indicating an essential role for SPOC1 in the maintenance of normal spermatogenesis. Whether SPOC1 functions in the self-renewal, proliferation and/or differentiation of spermatogonia is currently under further investigation.

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V. Immune cells: Targets of pathogenesis and therapy

Induction of an epitope-specific cellular immune response against SV40-induced mammary carcinomas in transgenic mice

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Tumor-specific cytotoxic T lymphocytes acquire tolerance or anergy early during tumorigenesis resulting in the failure of the immune system to control tumor outgrowth. Overcoming tumor-specific tolerance or anergy therefore is one of the most pressing prob-

lems in tumor immunology. Here, we studied tumor antigen-specific cellular immune responses in BALB/c mouse based WAP-T and WAP-T-NP transgenic mice in which the SV40 early gene region drives mammary carcinogenesis. WAP-T mice express the weakly immunogenic SV40 T-antigen (T-Ag), WAP-T-NP mice a chimeric antigen (T-NP-Ag) containing the strongly immunogenic epitope ($NP_{118-126}$) of the lymphocytic choriomeningitis virus (LCMV). Immunization with SV40 or a replication-competent recombinant vaccinia virus encoding SV40 T-Ag induced protective cellular immunity against transplantable SV40-transformed mKSA tumor cells only in naïve, virgin BALB/c mice, but not in virgin WAP-T or WAP-T-NP mice, suggesting that WAP-T and WAP-T-NP mice had acquired tolerance/anergy against SV40 T-Ag. In contrast, infection of WAP-T-NP mice with LCMV or with a recombinant vaccinia virus encoding the LCMV nucleopotein induced a strong $NP_{118-126}$-specific cellular immune response. The rapid kinetics of the $NP_{118-126}$-specific T cell response following infection of WAP-T-NP mice with LCMV suggests that in WAP-T-NP mice an endogenous NP_{118}-specific T cell response is primed during tumorigenesis, which is boosted by infection. We conclude that $CD8^+$ T cells specific for weakly immunogenic tumor specific epitopes are tolerized/anergized during tumorigenesis, while $CD8^+$ T cells specific for strongly immunogenic epitopes are primed and can be re-activated. The finding might open new avenues for boosting tumor-specific $CD8^+$ T cell responses.

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Manipulation of cellular activation pathways by CD33-related Siglec family members

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Siglecs *(sialic acid-binding immunoglobulin-like lectins)* belong to the Sialic acid-recognizing IG-superfamily lectins. They consist of an amino-terminal V-set immunoglobulin domain, which binds to sialic acid, and a variable number of C2-set immunoglobulin domains. Most Siglecs have an ITIM *(immunoglobulin receptor family tyrosine-based inhibitory motif)* and/or ITIM-like motif with which they are proposed to block cellular activation. Siglecs are prominently expressed on immune cells, mainly of the leukocyte and the macrophage lineage. However, there are great species-specific differences in Siglec expression which have been implied to contribute to the susceptibility of different species to AIDS development during the course of lentiviral infection.

So far, we confirm that different members of the Siglec family are able to suppress cellular activation of human Jurkat T-cells and primary T-cells because mutation of the ITIM abolished their inhibitory function. However unexpectedly, mutation of the ITIM not only diminished the ability of different Siglecs to inhibit cellular activation, but also lead to a dramatic increase in NFAT activation and the expression of T-cell activation markers.

Thus, Siglecs are able to trigger cellular activation under certain circumstances, which might be dependent on the recognition of “foreign” or “self” sialic acids being expressed on cellular membranes as well as on the surface of a variety of pathogens.

The karyopherin CRM1 is required for dendritic cell maturation

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Dendritic cells (DC) are the most potent antigen presenting cells (APC) of the immune system and are specialized to activate T as well as B cell-dependent immune responses. Mature DC are characterized by expression of CD83, a surface molecule that has been postulated to be required for efficient DC activity. Here we showed, that Leptomycin B (LMB), a highly specific inhibitor of the nuclear export receptor CRM1, abrogates the ability of DC to stimulate T cells in an allogeneic mixed lymphocyte reaction. Interestingly, this effect correlated with down-regulation of CD83, CD80 and CD86 surface expression during DC maturation, whereas other investigated DC surface molecules, such as MHC class I and II molecules were not significantly affected. Analysis of RNA distribution revealed that particularly the stimulated expression of CD83 depended on a functional CRM1 export receptor. Taken together, these data showed a critical involvement of the CRM1 transport receptor in DC maturation, most likely by enabling efficient nucleo-cytoplasmic translocation of specific mRNAs. Thus, interference with this pathway may provide new strategies to modulate DC function and, subsequently, DC-mediated immune responses.

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Phosphorylation of the HuR ligand APRIL by casein kinase 2 regulates CD83 expression

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Fully mature dendritic cells (DC) and, to a lesser extent, activated T and B cells express CD83, a surface molecule that appears to fulfill an important role in efficient T cell activation. Recently it has been shown that CD83 mRNA is transported from the nucleus to the cytoplasm by an uncommon route, involving the cellular RNA-binding protein HuR and the nuclear export receptor CRM1. Moreover, the shuttle phosphoprotein APRIL (ANP32B) has been shown to be required for HuR-mediated nucleocytoplasmic translocation of the CD83 mRNA by acting as an adaptor that links HuR and CRM1. Here, we were able to report that casein kinase 2 (CK2) phosphorylates APRIL on residue threonine244 (Thr^{244}) and demonstrated that the CK2-specific inhibitor TBB abolished CD83 expression in activated Jurkat T cells by interfering with the nucleocytoplasmic translocation of CD83 mRNA. Depletion and knock down studies demonstrated that the CK2 α' subunit is necessary for this regulation, while the CK2 α subunit seems to be dispensable. Taken together, these data significantly extend our knowledge of the complex regulation of CD83 mRNA processing and provides a novel strategy to interfere with CD83 expression.

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VI. New tools for research and diagnosis

Detection of specific structures in systems microscopy

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A big challenge in ultra-structural research based on EM imaging is the pinpoint localization of defined functional structures like macromolecules or macromolecular complexes, embedded in the complex molecular matrix of the cellular plasma. Moreover, the lifelike preservation of this function-driven molecular matrix is absolutely essential for a successful correlation of structural and functional cellular processes. Different preparation steps necessary for EM-imaging like chemical fixation, total dehydration and embedding in resins followed by polymerization at 60°C represent a bundle of artificial manipulations of the "system cell" and its structural components from outside far from biological functionality.

It was shown that these manipulations can massively alter the ultrastructural architecture of the cell, producing typical artefacts on the macromolecular level, thereby making the correlation between structural and functional units more difficult and in some cases even impossible. A solution to this problem is the use of cryo-techniques, which preserve the ultrastructural matrix in its functional state and topology as well as the molecular structure of the single macromolecules and thus their antigenicity, obligatory for successful detection or labelling. However, even with a perfect preservation on the ultrastructural level, the problem of identifying certain molecules – not organized in morphologically defined structures and thus directly recognizable – in a complex molecular matrix (ca. 1 x 1012 molecules/cell) still persists. The conventional solution is to detect the target molecules on ultrathin sections with nanogold conjugated antibodies visible as electron dense dots in the transmission electron microscope. On the next higher complexity level the same problem still persists: How can we differentiate morphologically undistinguishable cell types in a complex tissue? Or in the case of a homogeneous cell population: How to distinguish infected from non-infected cells? For this comparable systemic labelling protocols are essential. The ability to discriminate between different cells, tissues or organ regions without specific morphological characteristics is a problem on all complexity levels. This problem can only be solved with the help of specific markers detectable in all imaging devices (MRI, CLSM, REM and TEM). Due to their versatile physical and biochemical properties nanoparticles are suitable candidates to overcome the limits of classical labelling and enable a novel systemic approach in biomedical imaging.

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High-speed intravital microscopy visualizes the uptake of nanocrystal-labelled lipoproteins in liver cells in vivo

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Nanosomes are a new tool to study postprandial lipoprotein metabolism by multi-modal imaging in vivo. Here the lipid core of triglyceride-rich lipoproteins (TRL) was labelled with fluorescent nanocrystals to visualize hepatic lipoprotein uptake in vivo by high-speed intravital microscopy. Lipids of TRL were extracted and mixed with nanocrystals. Micelles were formed to obtain nanocrystal labelled TRL, designated nanosomes. Alternativly, human TRL and LDL were labelled with DiI. Mice were anaesthetised, a tail vein catheter was inserted and a liver lobule was prepared via a small incision. The liver uptake of injected nanosomes, TRL and LDL was followed by high-speed intravital confocal and multiphoton microscopy. The results were correlated with electron microscopy (EM). Liver binding and subsequent processing and cellular uptake of nanosomes and lipoproteins could be followed with a time resolution of 25 frames per second. After injection the lipoproteins were bound within five minutes in the space of Disse. In contrast to LDL, which was predominantly taken up by hepatocytes, for DiI-labelled TRL and nanosomes a significant and immediate uptake by stellate cells could be visualized. This uptake was confirmed by EM. The combination of confocal and multiphoton intravital microscopy with EM allows high-speed imaging of the TRL uptake in an improved time resolution. Thereby, the direct internalization of TRL components by hepatic stellate cells could be observed.

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In vivo evaluation of antibody-iron oxide nanoparticles in clinically relevant xenograft models of human cancer in immunodeficient mice and subsequent ultrastructural analysis by systemic microscopy

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Clinically relevant xenograft models are necessary to validate novel imaging tools before being applied in humans. They will be used to investigate the in vivo behaviour of antibody-conjugated iron oxide nanoparticles. To this aim human colon, breast, small cell lung cancer, neuroblastoma and malignant melanoma cells have been transplanted into immunodeficient mice to establish models for different tumour entities, with unique characteristics like vascularisation or antigen expression. For the optimization and in vivo validation of targeted iron oxide nanoparticles advanced electron microscopy techniques are essential. Therefore, a systemic investigation chain has been established allowing to characterize functionalized nanoparticles in vitro and follow their biological fate in vivo. The resulting tumours and possible metastases will be detected with immunohistochemistry and dedicated mouse coils in a clinical MRT scanner using targeted iron oxide nanoparticles. The amount and tissue distribution of these nanoparticles will be investigated by systemic microscopy. Therefore, tumour samples will be taken with a fine-needle high velocity biopsy gun and will undergo a bundle of special preparation techniques within a systemic investigation chain. The hydrated tissues will be directly analysed in the environmental SEM and subsequently in detail at molecular resolution by cryo-TEM, in order to detect uptake and spatial distribution of targeted nanoparticles within the tumour.

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Cellular prion protein localizes to the nucleus and is required for blood glucose regulation

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Cellular Prion Protein (PrP^C) is best known as the precursor of PrP^{Sc} (Sc- scrapie) that leads to transmissible spongiform encephalopathies. Several cellular processes have been purported for this protein, but remarkably little is known of the cellular function of the PrP^C. In a recent study we demonstrated that long term hyperglycaemia was associated with β-cell specific changes in PrP^C expression and aggregation. These findings suggested that PrP^C could play a direct role in β-cell function associated with blood glucose regulation. Here we demonstrate that mice deficient in PrP^C have a delayed response to hyperglycaemia. The impaired blood glucose regulation does not result from insulin deficiency or from insulin resistance suggesting that PrP^C loss impacts glucoregulation by other means. Furthermore, we show for the first time that PrP^C is most abundant in the nucleus *in vivo* where it interacts with lamin B1 and histone H3 raising the possibility that PrP^C functions at the epigenetic level. In conclusion, we demonstrate here that PrP^C is a novel target molecule that is required for proper response of β-cells to hyperglycaemia. A further understanding of the role of PrP^C in regulating islet cell function will provide valuable insight for blood glucose regulation. In addition, the identification of PrP^C as a nuclear protein provides new prospects in the elucidation of molecular mechanisms leading to neurodegeneration in prion diseases.

A preclinical model for monitoring treatment and prevention of metastasis in mammary carcinoma

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Breast cancer is the most frequently diagnosed type of cancer and the second leading cause of death among women in the Western world. Once localized breast tumors spread to other organs (metastasis), breast cancer is often incurable. The WAP-T and WAP-T x WAP-mutp53 mice developed in our lab adequately model the slow onset and development of human breast cancer from ductal carcinoma in situ (DCIS) up to invasive growth with metastasis. With these mice we aim to mirror the clinically relevant situation of metastatic disease that arises after excision of the primary tumor.

We have established a cell line from an endogenous, undifferentiated mammary carcinoma of a bi-transgenic WAP-T-WAP-mutp53 mouse which exhibits characteristics of tumor stem cells (G-2 cells). Orthotopic transplantation into syngeneic immune-competent mice of < 100 G-2 cells leads to the development of tumors with similar characteristics as primary tumors in WAP-T mice. Tumors arising from transplanted G-2 cells into WAP-T mice dissemination into a variety of tissues, blood and bone marrow. Disseminated tumor cells can be detected

with a sensitivity of less than 1 in 10^5 cells. Some transplanted animals develop visible lung metastases already within the period of tumor growth. To mimic the situation encountered by human mammary carcinoma patients, primary tumors arising from transplanted G-2 cells are surgically removed at an early stage to allow more time for disseminated cells to establish second site malignancies, which in human patients can take up to ten years. This preclinical model can be used to test various treatment regimens for the elimination of disseminated tumor cells and thus for prevention of metastasis in collaboration with clinical partners. To further refine the analysis, state of the art in vivo imaging of mice will be performed in collaboration with Dr. F. Alves, University of Göttingen, Germany.

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Die Kaufmännische Abteilung

Leitung: Dr. Volker Uhl

Die Neuausrichtung der Kaufmännischen Abteilung konnte in 2009 abgeschlossen werden. In der Stellungnahme der Gutachtergruppe der Leibniz-Gemeinschaft wurde die Effizienz der Kaufmännischen Abteilung besonders hervorgehoben; weitere Kosten und Leistungsoptimierungen können aus internen Veränderungen nicht mehr erzielt werden. Dementsprechend empfiehlt der Senatsausschuss Evaluierung zur weiteren Verbesserung der Servicefunktionen eine zunehmende Vernetzung administrativer Dienstleistungen mit den anderen Instituten des LCI-Verbundes. Durch Poolbildung von Verwaltungsbereichen zwischen den LCI-Instituten sollten Service-Units geschaffen werden, die einerseits eine höhere fachspezifische Professionalisierung erreichen und andererseits aufgrund des höheren Arbeitsaufkommens eine wirtschaftlichere Verteilung der Verwaltungskosten ermöglichen.

Nachdem in der Administration der Lohn- und Gehaltsabrechnung ein externer Dienstleister mit einem hohen verwaltungsspezifischen Know-How und geringen allgemeinen Verwaltungskosten beauftragt wurde, erscheint eine Zusammenlegung der verbleibenden Personaladministration mit anderen Verwaltungseinheiten von norddeutschen Forschungsinstituten keine wirtschaftlichen Vorteile zu gewähren.

Ebenso scheint eine Fusionierung des Finanz-Rechnungswesens mit ähnlichen Einrichtungen anderer Institute aufgrund des hohen Leistungsstandards und der intensiven Dienstleistungsorientierung dieses Bereichs keine Effizienzsteigerungen zu bieten. Auch erfordert die Drittmittelberatung, die Reisekostenabrechnung und das Haushaltscontrolling eine enge kommunikative Verbindung mit den wissenschaftlichen Einheiten des Instituts.

In 2010 sollte allerdings die strikte Trennung zwischen der kaufmännischen und der wissenschaftlichen EDV aufgegeben werden. Da die Schnittstellen zwischen den beiden Dienstleistungseinheiten in den letzten Jahren stetig angestiegen sind und die fachlichen Problemstellungen sich in vielen Aufgabenbereichen angenähert haben, wird eine organisatorischen Zusammenführungen beider Einheiten zukünftig angestrebt.

Des Weiteren ist eine organisationsübergreifende Vernetzung der Beschaffungsaktivitäten zwischen den norddeutschen LCI-Instituten zu prüfen. Nachdem am HPI ein elektronisches Einkaufssystem eingerichtet wird, sollten in einer Wirtschaftlichkeitsanalyse die Übertragbarkeit einer solchen integrierten Einkaufslösung untersucht werden. Durch die organisatorische Verbindung der Beschaffungseinheiten der norddeutschen LCI-Institute könnten einerseits Prozesskosten aber auch Einstandskosten für die beschafften Materialien und Dienstleistungen gesenkt werden.

Institutionelle Förderung und Drittmittel 2009 (vorläufiger Endstand 08. 01. 2010)

	Einnahmen	**Ausgaben**			
		Personalkosten	Sachkosten	Investitionen	**Gesamtwert**
Institutionelle Förderung	9.221.125 €	4.858.100 €	2.753.465 €	2.172.880 €	**9.784.445 €**
Z-Projekte	2.601.193 €	1.419.651 €	850.689 €	0 €	**2.270.340 €**
SB-Projekte	400.137 €	158.020 €	173.638 €	0 €	**331.658 €**
P-Projekte	0 €	0 €	0 €	0 €	**0 €**
Summe Drittmittel	3.001.330 €	1.577.671 €	1.024.327 €	0 €	**2.601.997 €**
Gesamtes HPI	**12.222.455 €**	**6.435.771 €**	**3.777.791 €**	**2.172.880 €**	**12.386.442 €**
davon:					
Bereich Grundfinanzierung	9.221.125 €	1.127.484 €	484.566 €	0 €	**1.612.050 €**
Bereich Gebäude und Technik	0 €	262.991 €	947.445 €	502.185 €	**1.712.621 €**
Bereich Wissenschaft	0 €	3.467.625 €	1.321.454 €	1.670.695 €	**6.459.774 €**
Summe Institutionelle Förderung	9.221.125 €	4.858.100 €	2.753.465 €	2.172.880 €	**9.784.445 €**
Internationale Förderungen	29.550 €	27.897 €	55 €		**27.952 €**
Europäische Förderungen	109.583 €	73.850 €	123.447 €		**197.297 €**
Bundesförderungen	960.185 €	450.342 €	502.699 €		**953.041 €**
Dt. Forschungsgem. SPP	97.294 €	70.550 €	20.541 €		**91.091 €**
Dt. Forschungsgemeinsch.	356.331 €	265.698 €	73.112 €		**338.811 €**
Deutsche Krebshilfe	510.365 €	307.191 €	93.033 €		**400.224 €**
Josè Carreras Stiftung	156.750 €	79.138 €	24.987 €		**104.125 €**
Wilhelm Sander-Stiftung	72.500 €	63.193 €	18.491 €		**81.684 €**
Förd. durch weitere Stiftungen	263.961 €	104.864 €	45.853 €		**150.717 €**
Industriekooperationen	282.934 €	84.170 €	48.111 €		**132.281 €**
diverse Mittelgeber	161.878 €	50.779 €	73.997 €		**124.776 €**
Summe Drittmittel	3.001.330 €	1.577.671 €	1.024.327 €	0 €	**2.601.997 €**

Personalentwicklung 2009

	Beschäftigung pro Programmbereich				HPI Gesamt	
	Virus-Wirts-Wechselwirkung		Zelluläre Dysregulation			
Wissenschaftliches Personal (davon Drittmittel)	**45**	**(19)**	**39**	**(19)**	**84**	**(38)**
Gastwissenschaftler (davon Drittmittel)	**12**	**(9)**	**11**	**(6)**	**23**	**(15)**
Stud. Hilfskräfte, Aushilfen (davon Drittmittel)	**1**	**(0)**	**1**	**(1)**	**2**	**(1)**
Stipendiaten (davon Drittmittel)	**3**	**(2)**	**3**	**(2)**	**6**	**(4)**
Diplomanden (davon Drittmittel)	**5**	**(0)**	**6**	**(0)**	**11**	**(0)**
Praktikanten (davon Drittmittel)	**6**	**(0)**	**5**	**(0)**	**11**	**(0)**
Summe (davon Drittmittel)	**72**	**(30)**	**65**	**(28)**	**137**	**(58)**

Erläuterungen: Stichtagerhebung 08. 01. 2010 / Anzahl der Köpfe

Es war im Jahr 2009 erstmalig möglich, eine prozentuale Aufteilung des wissenschaftlichen Personalbestands am Heinrich-Pette-Institut auf die jeweiligen Programmbereiche vorzunehmen. Zusätzlich zum wissenschaftlichen Personal waren 35 Personen im administrativen Bereich und zur technischen Unterstützung tätig.

HPI

Heinrich-Pette-Institut für Experimentelle Virologie
und Immunologie an der Universität Hamburg

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Dr. R. Greve

Wiss. Beirat
Vorsitzender
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Öffentlichkeitsarbeit
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Vorstand
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Stv. Wissenschaftl. Direktor
Prof. Dr. J. Hauber
Kaufmännischer Leiter
Dr. V. Uhl
Stv. Kaufmännischer Leiter
I. Jackstien

Kollegium
Vorsitzender
Prof. Dr. J. Hauber
Stv. Vorsitzender
Dr. H. Hohenberg

Kaufmännische Abteilung
Leiter
Dr. V. Uhl
Personal
K. Gessler
Finanz- und Rechnungswesen
S. Bräu
Einkauf
K. Krauß
I. Jackstien
Technische Dienste
C.-H. Lohm

Abteilungen, Forschungs- und Nachwuchsgruppen

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Molekulare Virologie
Leiter
Prof. Dr. T. Dobner

Forschungsabteilung
Allgemeine Virologie
Leiter
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Forschungsabteilung
Zellbiologie und Virologie
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Forschungsgruppe
Elektronenmikroskopie und Mikrotechnologie
Leiter
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Forschungsgruppe
Molekulare Pathologie
Leiterin
Dr. C. Stocking-Harbers

Betriebsrat
Vorsitzender
O. Nehls

Nachwuchsgruppe
Zelluläre Virusabwehr
Leiter
Dr. A. Grundhoff

Nachwuchsgruppe
Virus-Pathogenese
Leiter
Dr. M. Schindler

Nachwuchsgruppe
Influenza-Pathogenese
Leiterin
Dr. G. Gabriel

Gleichstellungsbeauftragte
A. Iwanski

Allgemeine Dienste
Biologische Sicherheit
Dr. A. Grundhoff
EDV
Dr. N. Zangenberg
H. Juretzka
Tierhaltung
H. Münd
Großgeräte
A. Düsedau
Zentrale/Empfang
P. Plarre
Laborküche
R. Karalic

Organigramm, Stand: Januar 2010

HPI

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