Steering Committee:

- Jean-Christophe AUDONNET
- David HEYMANN
- Quiang LIU
- Cornelis MELIEF
- Massimo TOMMASINO
- Guy VERNET
- Fabien ZOULIM

This meeting was made possible through educational grants from CLARA, Finovi and Lyonbiopôle.
Dear Participant,

It is our pleasure to welcome you to the symposium entitled:


in Fondation Mérieux’s Conference Centre “Les Pensières.” We hope you will enjoy this meeting, which brings together some of the world’s foremost experts.

The format of the discussion is intended to generate discussion and interaction among participants and to foster the dissemination of new information on this topic. The conference will provide an opportunity for specialists to exchange their knowledge and experience through collaboration with researchers from around the world.

Over the next three days, the team at Les Pensières will be on hand to help you with any questions you may have and to make your stay and conference as comfortable and valuable as possible.

Guy Vernet  
Scientific Director  
Fondation Mérieux

Fabien Zoulim  
Scientific Director  
INSERM Unit 871

For more information: www.fondation-merieux.org
## Scientific Programme

### Sunday 17 January 2010

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### Opening Session

18.15 - 19.30  
**Chaired by Christian Bréchot & Massimo Tommasino**

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<td>Hepatitis B and C - related liver carcinogenesis: paradigm for viral related human cancers</td>
<td>Christian BRECHOT, Institut Mérieux</td>
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<td>18.45</td>
<td>How do retroviruses induce cancers?</td>
<td>Ann SKALKA, Fox Chase Cancer Centre</td>
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<td>19.30</td>
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### Monday 18 January 2010

**Session 1**

08.30-10.00  
**Epidemiology, public health, and pathobiology of virally-induced tumors**  
**Chaired by Christian Trépo & Guy Vernet**

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<td>Epidemiology of virus induced cancers: HPV/immune suppression setting (HIV, HHV8, EBV)</td>
<td>Silvia FRANCESCHI, IARC</td>
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<td>08.50</td>
<td>Cervical cancer screening in low-resource countries</td>
<td>Catherine SAUVAGET, IARC</td>
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<td>09.10</td>
<td>Chronic viral hepatitis as a cause of hepatocellular carcinoma</td>
<td>Michael KEW, University of The WitWatersrand</td>
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<td>09.30</td>
<td>Clinical, Genetic and Molecular Epidemiology of HHV-8/KSHV infection and in vivo Clonality of Kaposi sarcoma</td>
<td>Antoine GESSAIN, Institut Pasteur</td>
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### Session 2
**Animal viruses and cancers**  
Chaired by Jean-Christophe Audonnet & Ab Osterhaus

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| 10.30 | Animal health | Hepadnavirus-induced hepatocellular carcinoma in animals | Marie-Annick BUENDIA  
Institut Pasteur |
| 10.50 | Animal health | Feline leukaemia virus: lessons and insights from a naturally occurring retrovirus and its associated diseases | James NEIL  
University of Glasgow |
| 11.10 | Animal health | Mastomys coucha, a natural rodent model to study papillomavirus-mediated skin carcinogenesis | Franck RÖSL  
German Cancer Research Centre |
| 12.00 | Lunch | | |

### Session 3
**Tumor mechanisms 1: oncogenes**  
Chaired by Antoine Gessain & Renaud Mahieux

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| 13.30 | Molecular mechanisms of EBV oncproteins in human carcinogenesis | Elliot KIEFF  
Harvard University |
| 13.50 | Insights into the molecular oncogenic mechanisms of herpesvirus-induced lymphomas in chicken | Venugopal NAIR  
Institute for Animal Health |
| 14.10 | HTLV-1 Tax, NF-κB deregulation, cell cycle perturbation, and cellular senescence | Chou-Zen GIAM  
Uniformed Services University |
| 14.30 | The role of viral signal-transducing membrane proteins in the life cycle of Kaposi Sarcoma Virus | Thomas SCHULZ  
Hannover Medical School |
| 15.15 | Coffee Break & Posters Session | Simone Mérieux Meeting Room |
Session 4  Tumor mechanisms 2: epigenetic  
16.15 - 17.00  Chaired by Zdenko Herceg & Thomas Schulz

16.15 - 16.35  Epigenetic signature as molecular marker for the detection of HPV-induced severe dysplasia and cervical carcinoma  
Alfred HANSEL  
Klinik für Frauenheilkunde und Geburtshilfe

16.35 - 16.55  Genome methylation and histone acetylation regulation in chronic HBV and HCV infections: impact on hepatocyte transformation  
Massimo LEVRERO  
University of Rome

16.55 - 17.15  Telomerase and telosomic dysregulation during HTLV induced carcinogenesis  
Eric WATTEL  
Hôpital Edouard Herriot

Posters Session (suite)  
17.30 - 19.30  Chaired by Birke Bartosch & Pierre Jalinot

17.30 - 18.30  Posters Session  
Simone Mérieux Meeting Room

18.30 - 19.30  Panel Discussion of Posters  
Plenary Session

19.30  Dinner

Tuesday 19 January 2010

Session 5  Tumor mechanisms 3: tumor suppressors  
08.30 - 09.45  Chaired by Pierre Hainaut & Massimo Levrero
Scientific Programme

### Session 6
**Tumor mechanisms 4: cell signaling**
Chaired by Kuan Teh Jeang & Vincent Lotteau

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<td>HBV X protein-mediated dysregulation of p53-induced apoptosis</td>
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<td>Role of microRNAs and tumor suppressors in HTLV induced cell growth dysregulation</td>
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<td>How does HTLV-1 persist in vivo?</td>
<td>Charles BANGHAM</td>
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<td>10.05 - 10.25</td>
<td>Molecular mechanisms of HPV-mediated TLR9 down-regulation</td>
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<td>Hepatitis C virus induced modification of hepatocyte metabolism and signalling: impact on viral persistence and tumor promotion</td>
<td>Ralf BARTENSCHLAGER</td>
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**Prevention of cancer**
Chaired by Thomas Baumert & Jack Wands

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<td>Ding-Shinn CHEN</td>
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<td>Geneviève INCHAUSPE</td>
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<td>Next generation of HPV prophylactic vaccines</td>
<td>Lutz GISSMANN</td>
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<td>12.00 - 12.20</td>
<td>Impact of antiviral treatment of chronic hepatitis B and C on hepatocellular carcinoma prevention</td>
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# Scientific Programme

## Session 8

**Therapy of virally induced cancers**

**Chaired by Christophe Caux & Philippe Merle**

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<td>Viruses as tools: oncolytic viruses for the therapy of cancers</td>
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## Final Session

**Chaired by Cornelis J. Melief & Massimo Tommasino**

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<td>Why do viruses cause tumors? New technologies to human tumor virology</td>
<td>Patrick S. MOORE</td>
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<td>16.05 - 16.15</td>
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Opening Session
How do retroviruses induce cancer?

Ann SKALKA
Fox Chase Cancer Center - USA

It has been more than 100 years since it was first appreciated that (retro)viruses can cause cancer. We now know that retroviruses are genomic parasites; their replication depends on a unique ability to insert a DNA copy of their genome into the chromosomes of the cells that they infect. This property, together with subsequent co-option of host cell transcription and translation machineries, is key to understanding mechanisms by which retroviruses can induce malignancies, an unfortunate outcome that is not a requirement for their propagation. «Accidents» that occur during normal steps in the retrovirus life cycle can lead to the generation of rapidly transforming, oncogene-transducing virions, or tumorigenesis after medium or long latency. Knowledge gained through study of such mechanisms provides a useful starting point for new insights into tumor virology.
Session 1

Epidemiology, public health impact, and pathobiology of virally-induced tumors
Epidemiology of virus induced cancers: HPV/immune suppression setting (HIV, HHV8, EBV)

Silvia FRANCESCHI
IARC - France

Around the world, infection is one of the most important causes of cancer. Almost one in every five malignancies can be attributed to infectious agents. Among infection-related neoplasms, cancers of the stomach, liver and cervix uteri detain the highest incidence figures, and are known to be largely attributable to *Helicobacter pylori*, hepatitis B and C viruses, and human papillomavirus (HPV), respectively. Other infectious organisms can also cause cancer; these include the Epstein-Barr virus (nasopharyngeal carcinoma, and different types of lymphoma), human herpes virus-8 (Kaposi sarcoma, KS), human T-cell leukemia virus type I (leukaemia, lymphoma), liver flukes (cholangiocarcinoma) and schistosomiasis (bladder cancer).

HIV infection is strongly associated with an elevated risk at many cancer sites. HIV, however, is not carcinogenic per se and increases the risk of many virus-associated cancers, mainly via immunodeficiency. The specific role of immunodepression was supported by a recent meta-analysis showing similar elevated risk among solid organ transplant recipient. Whereas highly active antiretroviral therapy (HAART) introduction has curbed the incidence of cancer associated with very severe immunodeficiency (i.e., KS and brain and immunoblastic non-Hodgkin lymphoma), it has not yet shown a beneficial effect towards ano-genital cancers associated with HPV. Furthermore, HAART-induced improvement of life expectancy may ultimately increase the cancer burden in HIV-infected individuals.
Cervical cancer screening in low-resource countries

Catherine SAUVAGET
IARC - France

Cervical cancer is diagnosed in 500,000 women every year and leads to 270,000 deaths. It is the main cause of morbidity and premature death in middle aged women from low-resource countries. Eighty-five percent of deaths from cervical cancer occur in sub-Saharan Africa, Latin America, and South Asia. Cervical cancer is due to the persistent infection of the cervix by the human papillomavirus (HPV).

Secondary prevention of cervical cancer relies on screening and early detection of precancerous lesions. In high-resource countries, screening programmes by Pap smear have shown to be effective in reporting a decrease in the number of new cancer cases and in diagnosing and treating asymptomatic precancerous lesions.

Unfortunately, Pap smear screening programmes are scarce in low-resource countries and its success relies on adequate human resources, high quality smears, adequate laboratories and health infrastructures, high screening coverage and follow-up. However, recent studies performed in India reported that alternative screening methods such as visual inspection with diluted acetic acid (VIA) performed similarly to Pap smear in terms of specificity and sensitivity and demonstrated a significant decrease in incidence and mortality from cervical cancer. Another screening alternative, which can be used as a triage test, is the HPV-DNA testing to detect any viral infection of the cervix.
Chronic viral hepatitis as a cause of hepatocellular carcinoma

Michael KEW
University of the Witwatersrand - South Africa

Chronic hepatitis B (HBV) and hepatitis C (HCV) virus infections are major causes of hepatocellular carcinoma (HCC), and delta hepatitis virus (DHV) co-infection increases the hepatocarcinogenic potential of HBV. Other viruses claimed to cause chronic hepatitis, viz. hepatitis G virus, TT virus, and SEN virus do not cause HCC.

Chronic HBV infection is the major global cause of HCC, being responsible for over 50% of HCCs worldwide and for 80% of HCCs in the high incidence regions in the Asia-Pacific and sub-Saharan Africa. The infection that becomes chronic is mainly acquired in the early months or years of life, either perinatally or horizontally, and carries life-time relative risk of HCC development that may be as high as 100. In Chinese and Black African patients the tumour presents at a relatively young age. HBV-induced HCC carries a grave prognosis, with almost all patients dying within one year.

HBV-induced HCC usually develops in a cirrhotic liver, although this association may be less close in Black Africans.

A series of genetic and epigenetic changes, in association with hepatocyte proliferation, are involved in HBV-induced hepatocarcinogenesis. HBV DNA integrates into chromosomal DNA in the great majority of these tumours. The HBV x gene seems to play an important role in hepatocarcinogenesis although much still remains to be learnt about the exact mechanisms involved.

The incidence of chronic HCV infection and HCV-induced HCC has increased in a number of countries during recent decades, particularly in Japan and Egypt, but also in North America, Great Britain, parts of Europe, and Australia. The infection is largely acquired in adulthood as a result of intravenous drug abuse or sexual transmission. HCV-induced HCC therefore occurs at an older age than HBV-induced HCC. The association with cirrhosis and HCC development is closer in HCV-induced than in HBV-induced HCC. HCV does not integrate into chromosomal DNA. Much still remains to be learnt about the molecular genesis of HCV-induced HCC.

HDV infection always occurs in association with HBV infection. In those geographical regions where co-infection with HBV and HDV is common progression to hepatic dysfunction, cirrhosis, and HCC is accentuated.
Kaposi’s sarcoma (KS) associated herpesvirus (KSHV), also known as human herpesvirus-8 (HHV-8), is a γ2-herpesvirus considered as the etiological agent of all KS forms (classic, endemic, iatrogenic and epidemic). The worldwide prevalence of this tumor of lymphatic endothelium origin is increasing and the number of KS cases was estimated in 2002 to be around 65,000, or nearly 1% of all diagnosed cancer. This virus is also associated with rare lymphoproliferative disorders as primary effusion lymphoma, multicentric Castleman disease and associated leukemia.

Our laboratory has developed long-term studies in high HHV-8 endemic areas in order to get new insights into the clinical, genetic and molecular epidemiology of HHV-8 infection, as well as in vivo clonality of HHV-8 associated tumors.

In low endemic areas, as most of the European countries (except in the Mediterranean area) and North America, HHV-8 is mainly endemic among men who have sex with men, being transmitted during repeated sexual relationships. By studying large endemic populations in South America and Central Africa, we have demonstrated a high familial aggregation of HHV-8 infection with most infection being acquired during childhood and adolescence, and with no evidence for transmission among heterosexual couples. Thus, in high endemic areas, the major modes of transmission are from mother to child and between siblings (1,2,3). To investigate whether host genetic factors could explain some part of the familial aggregation, a segregation analysis was performed. Results provided evidence for a recessive gene controlling susceptibility/resistance to HHV-8 infection with a major effect during childhood (4).

Concerning molecular epidemiology, sequence analysis of the highly variable open reading frame K1 of HHV-8 has allowed the identification of five main molecular subtypes (A-E), with a non-random worldwide distribution mostly correlating with geography and host ethnic background. HHV-8 subtypes A and C prevail in Europe, Mediterranean countries and U.S.A, subtype B predominates in sub-Saharan Africa; subtype D is found in individuals from the Pacific area; subtype E is observed among Native American populations. We have developed studies in several populations having different genetic backgrounds and often living in remote areas including, Pygmies in Central Africa (5), Melanesians in Vanuatu (6), Amerindians in South America (7,8), Bouriats in Siberia (9)…
Our results confirm, in all cases, a clear molecular clustering of HHV-8 strains according to ethnic/geographic background. Furthermore, the genetic variability of K1 gene appears to be a good molecular tool to better understand ancient migrations of human population infected by such herpesvirus.

Lastly, we have also demonstrated, by studying the size heterogeneity of the HHV-8–fused terminal repeat region that some advanced KS tumor lesions are true monoclonal expansion of spindle cells (SC) infected by latent HHV-8, indicating that the virus was present in vivo prior to the expansion of a tumor clone of SC (10). This strongly sustains the etiological role of HHV-8 infection in Kaposi sarcoma in vivo. Furthermore, individual KS disseminated tumor skin lesions were found to represent distinct expansions of HHV-8–infected spindle cells. Thus, our results suggest that KS lesions, especially in patients with advanced skin tumors, are reactive proliferations (originating from different infectious events) rather than true malignancies with metastatic dissemination (11).

Session 2

Animal viruses and cancers
Hepadnavirus-induced hepatocellular carcinoma in animals

Marie-Annick BUENDIA
Institut Pasteur - France

The human hepatitis B virus (HBV) is the prototype member of the hepadnaviridae family of viruses that infect a limited number of mammalian species and birds. Hepadnaviruses share common structural and biological properties, including virion structure and genomic organization, replication via a reverse transcription step, preferential tropism for liver parenchymal cells, restricted host range, and ability to induce acute and chronic liver diseases. In the absence of convenient experimental systems for productive HBV infection, animal hepadnaviruses have provided useful tools for studying the hepadnavirus life cycle, the natural history of chronic viral infection, as well as for preclinical studies of anti-viral treatments. Overwhelming epidemiological evidence has linked chronic HBV infection to the development of hepatocellular carcinoma (HCC). This cancer is a rapidly increasing cause of death in many countries, and it remains refractory to current chemotherapeutic regimens. Moreover, HCC is frequently diagnosed when advanced stage of the disease precludes local ablative or surgical interventions. In this context, limitation of HBV spread and inhibition of HBV replication stand among the most efficient preventive strategies against HBV-related HCC. Advances in our understanding of HCC pathogenesis are needed to promote the clinical development of early tumor markers and novel targeted agents with improved efficiency. Among hepadnaviridae, the woodchuck hepatitis virus (WHV) and ground squirrel hepatitis virus (GSHV) have been causally related to liver carcinogenesis in their respective hosts. Infected animals develop chronic hepatitis and HCCs that are in many points similar to those associated with HBV infection in humans, although the liver of these rodent species is not susceptible to cirrhosis. In experimental inoculations shortly after birth, virtually all that chronic WHV carrier woodchucks develop HCCs with a median tumor-free survival of 24 months and a median life expectancy of 30-32 months, placing WHV at the first rank among known hepatocarcinogens. Most woodchuck HCCs carry integrated WHV sequences that may be detected by Southern blotting, reflecting clonal outgrowth of a transformed cell targeted by one or more integration events. Search for oncogenes at viral integration sites has led us to demonstrate that WHV acts mainly as an insertional mutagen of myc family genes. The highest insertion frequency found in the N-myc2 retroposon and in two nearby loci on the X chromosome called b3n and win.
The oncogenic potential of such integrations was demonstrated by the development of HCC in virtually all transgenic mice carrying WHV insertion sites in c-myc or N-myc. Thus, insertional mutagenesis of myc oncogenes confers a selective growth advantage on target hepatocytes, leading to the emergence of neoplastic nodules or providing an additional step in tumor progression. Other studies in the GSHV/ground squirrel model have evidenced a lower oncogenicity of this virus, and absence of insertional mutagenesis mechanisms. However, activation of Myc is frequently achieved via genetic amplification in ground squirrel HCCs.

In conclusion, animal models of hepadnavirus-related carcinogenesis have provided crucial tools for preclinical assessment of the efficacy and safety of antiviral drugs that can be used for chemoprevention of hepatocellular carcinoma. Moreover, unraveling the mechanisms of hepadnavirus-related hepatocarcinogenesis becomes increasingly important for the design of novel therapeutic approaches aimed at combating this deadly cancer.
Feline leukaemia virus: lessons and insights from a naturally occurring retrovirus and its associated diseases

James C. NEIL
University of Glasgow - UK

Feline leukaemia virus represents a paradigm for the natural history of γ-retrovirus infection in an outbred host species. Despite some successes in control and prevention of infection this virus remains a significant pathogen of its primary host, the domestic cat. The virus is horizontally transmitted but the outcome of exposure is not uniform and is subject to age-related resistance driven by immune maturation in the host. Outcomes vary from complete clearance of infection to persistent high-level viraemia and early death from degenerative and/or neoplastic diseases, while intermediate states of latent infection have also been described. The molecular pathogenesis of FeLV in the persistently infected host has been characterised in detail as an evolutionary progression where a relatively homogeneous infecting virus acquires increased pathogenic potential by processes including sequence duplication, point mutation and recombination with endogenous proviruses and host gene sequences. The high frequency of transduction of cellular genes by FeLV is a notable feature in malignant diseases associated with FeLV. The knowledge that the host immune response can successfully control FeLV infection has been translated to the produce the first commercial retrovirus vaccines. A number of conventional and recombinant vaccines have now been in field use for over a decade. FeLV has recently been found in significant disease outbreaks in large felids, demonstrating the ongoing risks of cross-species transfer with this virus family. Other examples are provided by koala retrovirus (KoRV) and the emerging evidence of XMRV as a human disease agent. Our detailed knowledge of FeLV acquired over many years may be of strategic value in addressing these new threats to human and animal health.
Mastomys coucha, a natural rodent model to study papillomavirus-mediated skin carcinogenesis

Frank RÖSL
German Cancer Research Center - Germany

The rodent Mastomys coucha is latently infected with Mastomys natalensis papilloma virus (MnPV). These animals are unique in spontaneously developing multiple benign skin tumors such as papillomas and keratoacanthomas, for which MnPV is the etiological agent. Previous studies demonstrated that MnPV persistence and viral load correlates with the development of skin tumors. We recently discovered a novel virus, Mastomys coucha papilloma virus 2 (McPV2), which is the first described rodent papillomavirus inducing anogenital lesions in its natural host. McPV2 has a similar tropism as MnPV, but is apparently less abundant in our colony. To investigate immunological events during infection, a variety of animals were tested for the presence of both virus types and humoral responses against viral proteins. Here, a strong correlation between high viral copy numbers and L1-specific antibodies predominantly in older, tumor-bearing animals could be detected. Interestingly, in contrast to the early proteins E6/E7, we found extensive antibody titers against E2 also in tumor-free animals, which were even higher than the serum responses against the L1 protein. Follow-up studies revealed that E2 seropositivity marks the latent infection state and precedes tumor formation. Mastomys coucha represents an excellent model to study molecular and immunological aspects of virally induced pathogenesis of the skin. Furthermore, these animals will serve as an in vivo system to investigate prophylactic and therapeutic approaches against papillomavirus caused epithelial tumors. In the meantime, we produced virus-like particles and currently test the efficiency of a prophylactic vaccine in the prevention of MnPV infections and the associated skin papillomas.
Session 3

Tumor mechanisms 1: oncogenes
Epstein-Barr Virus (EBV) Nuclear and Membrane Proteins Cause Perpetual Lymphocyte Proliferation

Elliot KIEFF
Harvard University - USA

Epstein’s discovery of the first human tumor virus in endemic African Burkitt Lymphomas in 1964 (Epstein, 1964) and ensuing discoveries that EBV infection efficiently causes limitless B-cell proliferation, in vitro, {Henle, 1967; Pope 1967}, in uninfected primates {Deinhardt, 1975; Shope, 1975}, and in immune deficient humans {Purtill, 1977} informed our long term objective of understanding the basic underlying mechanisms for EBV mediated B-lymphocyte transformation at the molecular and sub-molecular level. We began with the characterization of EBV structural proteins and genomes {Kieff, 1974}{Pritchett, 1975}. We then determined the extent of genome transcription and translation in latent B lymphocyte infection {Orellana, 1977}, derived complete EBV DNA restriction endonuclease maps from multiple strains {Given, 1978), identified the two types of EBV genomes, derived complete overlapping EBV DNA molecular clone sets {Dambaugh, 1980}, sequenced EBV transcripts from latently infected B lymphocytes, confirmed open reading frames to encode latency associated proteins using open reading frame-specific sera, identified the EBV genes and codons essential and non-essential for lymphocyte transformation using recombinant reverse genetics, identified interactive cell proteins, downstream signaling pathways through which EBV causes perpetual cell growth and survival, as well as EBV latent infection induced cell proteins, provided expression vectors for collaborations with the Moss, Klein, and Rickinson groups to elucidate human T-cell immune responses that contain latent infection associated B-cell proliferation, and derived a prototype EBV vaccine.

Principally, EBV has five genes that have essential roles in causing efficient B cell proliferation and survival. These genes encode 4 nuclear proteins, EBNA2, EBNALP, EBNA3A, and EBNA3C, which constitutively regulate EBV and cell gene expression in latent growth transforming infection, including the CD21, CD23, c-myc, and Tcl1 promoters through RBP-Jk, the key transcription factor in Notch signaling. EBV also encodes 2 integral membrane proteins, LMP1, which constitutively activates Toll, IL1, TNF signaling pathways. LMP2 constitutively activates B-cell receptor (BCR) signal transduction, desensitizes BCR signaling and inhibits reactivation from Latency and also enables transformation of not only mature B-lymphocytes, but immature B lymphocytes as well (latter work of Longnecker and Hammerschmidt groups). EBNA1 also has a key role in efficient EBV episome persistence and enhanced transcription, but does not directly effect cell transformation.
Ongoing experiments will be presented indicating that EBNA2, EBNALP, EBNA3A, and EBNA3C essential transformation functions are in constitutively and coordinately directly regulating cell gene transcription, including c-myc, p16INK (Maruo and Takada) and Tcl1. This regulation is, in large measure, but not exclusively, through interacting with specific RBP-Jk sites at various distances from cell promoters. EBNA2 is a strong activator, but unable to engineer H3K4 trimethylation in the absence of EBNALP, which associates strongly with a nuclear-cytoplasm shuttling factor HA95, and removes repressors from promoters targeted by RBP-Jk. LMP1 constitutively and coordinately interacts with EBNA regulation of cell growth by constitutively activating canonical and non-canonical NF-κB and Interferon signaling pathways (IRF7 activation by Pagano group), thereby increasing cell survival. LMP1 constitutive signaling is mediated by heteromeric interaction of transmembrane domains 1-2 with transmembrane domains 3-6. NF-κB activation is essential for EBV infected cell survival and inhibition of NF-κB activation causes cell death. We have therefore undertaken a genome wide cell screen for enzymes that are essential for LMP1 (TES2) mediated canonical NF-κB activation. These studies identify novel kinases, phosphatases, ubiquitin ligases, and ubiquitin proteases that effect canonical LMP1 induction of NF-κB activation.
Insights into the molecular oncogenic mechanisms of herpesvirus-induced lymphomas in chicken

Venugopal NAIR
Institute for Animal Health - UK

Oncogenic viruses are thought to account for about 20 per cent of all human cancers. In animals also, tumors induced by oncogenic viruses have been of major concern due to animal welfare issues and economic losses. The modern poultry industry is estimated to produce a massive 55,000 million chickens annually. Historically, neoplastic diseases induced by oncogenic viruses in chickens have been valuable tools in elucidating several important molecular pathways of oncogenesis. Herpesviruses include a large group of DNA viruses associated with a wide range of diseases in man and animals, including different types of cancer. Marek’s disease virus (MDV) is a highly contagious and oncogenic herpesvirus of chickens that costs the global poultry industry at least $US1bn per annum. Marek’s disease (MD), induced by MDV, is characterized by rapid-onset T-cell lymphomas in multiple visceral organs. MD is also one of the first examples of a successful widely used vaccine against any type of cancer. Although the use of vaccines by the poultry industry has helped to reduce the losses from the disease, the virus has continued to increase in virulence, necessitating the periodic introduction of new generations of vaccines.

In order to develop more effective, sustainable, vaccines, it is important to understand the molecular pathogenesis of MD and role of various viral determinants in inducing the disease. Using reverse genetics approaches on infectious clones of the viral genomes cloned as bacterial artificial chromosome (BAC), we were able to identify some of the viral proteins that are important in the induction of tumours. The most important viral gene that is associated with oncogenicity is the MDV-encoded leucine-zipper protein Meq, a 339-amino acid protein with an N-terminal basic leucine zipper domain and a C-terminal transactivation domain. Deletion of Meq gene totally abolished the oncogenicity of the virus. For further delineation of the Meq function, we have identified Meq-interaction proteins such as Fos-Jun and other AP-1 family proteins, C-terminal binding protein (CtBP), hsp-70 and others.

More recently, we have identified 14 novel microRNAs encoded by MDV. These miRNAs expressed as three clusters, are expressed at very high levels in MDV-induced tumours and hence thought to be important in the transformation of the target lymphocytes. Using miRNA-deletion mutants of MDV, we show that some of the miRNA clusters are very important in oncogenesis. The functions of MDV-encoded viral proteins and microRNAs can provide insights into the molecular pathways of virus-induced oncogenesis, and these information could also be used in designing novel vaccines.
HTLV-1 Tax, NF-κB Deregulation, Cell Cycle Perturbation, and Cellular Senescence

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HTLV-I is the causative agent of adult T-cell leukemia/lymphoma (ATL), a malignancy of CD4+ T cells whose etiology is thought to be associated with the viral trans-activator, Tax. We have shown recently that Tax can drastically up-regulate the expression of cyclin dependent kinase inhibitors, p27Kip1 and p21CIP1/WAF1, through protein stabilization and mRNA trans-activation and stabilization respectively. The surge in p21CIP1/WAF1 and p27Kip1 begins in S phase and leads to aberrant cell cycle progression that ends in cellular senescence. Importantly, HeLa and SupT1 T cells infected by HTLV-1 also arrest in senescence, contrary to the prevailing paradigm, which maintains that HTLV-1 induces proliferation of infected cells. New results will be presented to show that Tax causes the DNA replication licensing factor, Cdt1, to accumulate in S phase. The increased Cdt1 level in Tax-expressing cells correlates with DNA hyper-replication. Most Tax-expressing HeLa cells are slow to progress into mitosis and lag in S and G2 phases of the cell cycle with dramatically elevated levels of p27Kip1 and p21CIP1/WAF1 and reduced levels of cyclin B1 and Skp2. The use of HeLa/18x21-EGFP and HeLa-FUCCI—HeLa cell lines that expresses EGFP under the control of 18 copies of the Tax responsive 21-bp repeat element and fluorescent ubiquitin cell cycle indicators respectively—in time-lapse photography further reveals that many Tax-expressing cells eventually progress through S and G2, and enter into an irreversible state of cell cycle arrest, with some bypassing mitosis altogether. Indeed, cells that failed mitosis are enlarged with exaggerated nuclei and express senescence-associated β-galactosidase, consistent with mitotic defects and eventual entry into senescence. Interestingly, a small population of HeLa cells was found to progress into mitosis with high levels of Tax expression, suggesting that genetic or epigenetic changes can readily occur to circumvent Tax-induced senescence. Multiple subclones of HeLa/18x21-EGFP with variable degrees of resistance to Tax-induced senescence have now been isolated. Finally, evidence will be reported to indicate that Tax causes cellular senescence by disrupting the IKK/NF-κB/I-κBα auto-regulatory loop.
Session 4

Tumor mechanisms 2: epigenetic
Epigenetic signature as molecular marker for the detection of HPV-induced severe dysplasia and cervical carcinoma

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**Background:** The sensitivity of a single Pap-smear for the detection of dysplasia and cervical cancer is poor. By implementation of a HPV-DNA test in a primary screening setting the sensitivity approaches 100%. However, the specificity of the HPV-DNA test for the detection of high grade CIN, particularly for women below 30 years of age, is inadequate.

**Objective:** To define a methylation signature specific for high grade CIN and cancer (CIN3+) for the use in cervical cancer screening programs.

**Methods:** Cervical carcinogenesis is a multi-step process which also involves gene silencing. A common mechanism for silencing is hypermethylation of cytosines in CG dinucleotides of CpG islands, regions with high GC content predominantly located in the promoter region of genes. Hypermethylated genes can be detected with high sensitivity and specificity by the use of real-time methylation-specific PCR (MS-PCR). Based on previously performed cDNA array analyses genes which were consistently down-regulated in cervical cancers were selected. Further candidate genes were identified by CpG island arrays. In order to identify a subset of genes which is exclusively methylated in CIN3+, MS-PCR were performed for all of these genes using pools of DNA derived from normal cervical scrapes and cervical carcinomas. In a subsequent step the methylation status of all promising genes was evaluated using single samples only. The best candidate genes were then evaluated in a large series of well-defined clinical samples.

**Results:** Of 150 candidate genes evaluated most genes did not fulfill the stringent selection criteria required for a methylation signature. Most genes were methylated, albeit at low levels, in over 10% of HPV-negative cervical scrapes and were therefore not evaluated further. For the remaining six genes MS-PCR was performed on individual cervical scrapes taken from women who were HPV-DNA negative (n=77), HPV-DNA positive but colposcopically normal (n=90), HPV-DNA positive with histologically proven CIN3 (n=49), and HPV-positive with invasive cervical carcinoma (n=65). The sensitivity for the detection of CIN3 or cervical cancer based on the methylation of the single genes ranged from 52% to 78%. Sensitivity reached 92% if at least one of the six genes was methylated. Under these conditions specificity was well over 90%.
Conclusion/Outlook: We have identified a methylation signature characteristic for CIN3 and cervical cancer. One promising application would be to use MS-PCR as a reflex test for HR-HPV positive cases in a primary screening population. In this scenario the calculated sensitivity and specificity for CIN3+ would be 92% and >99%, respectively. The clinical utility of this signature is being tested in an ongoing prospective study.
Session 5

Tumor mechanisms 3: tumor suppressors
The hDlg tumour suppressor and related proteins are targeted by HPV E6 oncoproteins

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Cervical cancer-causing HPV types encode two major oncoproteins, E6 and E7, both of which are still required many years after the initial transforming events for maintenance of the transformed phenotype. Both viral proteins target critical regulators of the cell cycle and pro-apoptotic machinery, and frequently recruit components of the ubiquitin proteasome system in order to do this. An intriguing feature of the ‘high-risk’ E6 proteins is their possession of a PDZ binding motif, which directs their interactions with a number of cellular PDZ domain-containing substrates. These include hDlg and hScrib, both of which are putative tumour suppressors involved in the regulation of cell polarity and cell proliferation. Loss of these proteins appears to be a common feature of many different cancers, although why these proteins are targeted by HPV E6 remains uncertain. By targeted knockdown of hScrib and hDlg in keratinocytes, together with an analysis of how E6 interacts with these proteins, we are now beginning to establish how loss of hDlg and hScrib can contribute to the development of malignancy and also aid the normal viral life cycle.

In order to more fully identify common mechanisms of virally-induced tumorigenesis, we have also embarked upon an analysis of those HPV types associated with the development of skin cancers. Interestingly, we have identified a subset of protein targets that appear common to both the mucosal and cutaneous HPV types. In particular, the E6AP ubiquitin ligase appears central to the function of the cutaneous HPV E6 proteins. These results define a cellular target whose interaction appears to be common to all the HPV E6 proteins we have so far analysed, and which offers a potentially common route for therapeutic intervention.
HBV X protein-mediated dysregulation of p53-induced apoptosis

Ouriana ANDRISANI
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Chronic Hepatitis B Virus (HBV) infection is a major etiologic factor in pathogenesis of hepatocellular carcinoma (HCC). The HBV X protein (pX) is considered a weak oncogene or a co-factor in HBV-mediated hepatocarcinogenesis. However, the mechanism of pX-mediated hepatocyte transformation is not yet fully understood. We have shown that inhibition of Polo-like kinase 1 (Plk1) suppresses pX-mediated hepatocyte transformation. This is significant because, Plk1, required for mitotic entry and progression, is elevated in human cancers including HBV-HCC. More importantly, clinical trials are in progress targeting Plk1 inhibition for other types of cancer, suggesting Plk1 also could serve as therapy target for HBV-HCC. However, much remains to be understood about the role of Plk1 in oncogenic transformation.

We have found that pX promotes DNA re-replication-induced DNA damage and propagates DNA damage, resulting in polyploidy. Also, pX activates Plk1 in G2 phase of untransformed hepatocytes. In turn, activated Plk1 suppresses DNA repair, p53 apoptosis, and attenuates the DNA damage checkpoint, thereby propagating damaged DNA to subsequent cell generations. This is the first example demonstrating a molecular mechanism of checkpoint adaptation in mammalian cells.

Furthermore, we have identified by a genome-wide siRNA library screen two novel proteins, ZNF198 and SUZ12, whose depletion rescues pX-expressing hepatocytes from p53-induced apoptosis, suggesting that these proteins are tumor suppressors of pX-mediated transformation. Utilizing a novel in vitro cellular model of pX-mediated hepatocyte transformation, we found that as pX-expressing cells become oncogenically transformed the protein levels of ZNF198 and SUZ12 decrease, while protein levels of Plk1 increase. Elevated protein levels for Plk1 and reduced levels of ZNF198/SUZ12 are also observed in human HBV-HCC-derived cell lines. This inverse relationship between Plk1 and ZNF198/SUZ12 suggests that ZNF198 and SUZ12 mediate effects of Plk1 activation during pX-mediated hepatocyte transformation. These studies are likely to reveal new therapy targets and diagnostic markers for HBV-HCC, as well as for other human cancers that exhibit elevated expression of Plk1.


Role of microRNAs and tumor suppressors in HTLV induced cell growth dysregulation

Kuan-Teh JEANG
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It has been 30 years since the original identification of HTLV-1. During the past three decades, much has been learned about how the virus uses its Tax oncprotein to transform cells. In this presentation, I will discuss the subversion of cellular checkpoints and the activation of cellular proliferative factors by HTLV-1. One mechanism used by the virus to dysregulate cellular proliferation is through changes in the cell’s microRNA expression profile. I will also discuss emerging findings on how HTLV-1 may transform human progenitor/stem cells.
Session 6

Tumor mechanisms 4: cell signaling
How does HTLV-1 persist in vivo?

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The human leukaemia virus HTLV-1 persists in vivo by two different mechanisms. We showed that HTLV-1 spreads directly between cells by a specialized cell-cell contact called the virological synapse. During the chronic phase of infection, HTLV-1 persists in the host chiefly by driving proliferation of HTLV-1-infected T cell clones. This proliferation frequently results in a very high proviral load, which is strongly correlated with the risk of inflammatory diseases such as HTLV-1-associated myelopathy (HAM). In 5% of HTLV-1-infected individuals, one or more clones undergo malignant transformation, resulting in adult T cell leukaemia/lymphoma (ATLL). It has recently been suggested that ATLL is a tumour of "regulatory" CD4+ T cells, because ATLL cells frequently express several markers characteristic of regulatory T cells (CD4, CD25, FoxP3, CCR4, GITR).

We aim to identify and quantify the selection forces that determine the size of each HTLV-1-infected T cell clone and hence the risk of the inflammatory (HAM) and malignant (ATLL) diseases. I shall summarize evidence that the abundance of an HTLV-1+ T cell clone is determined by the balance between the host cell-mediated immune response and virus-driven T-cell proliferation. Specifically:

i) The genetically-determined efficiency of the cytotoxic T lymphocyte (CTL) response is a major determinant of proviral load and the risk of inflammatory diseases.

ii) HTLV-1 infection elicits a high frequency of FoxP3+ CD4+ T cells: this frequency correlates inversely with the efficiency of the CTL response to HTLV-1, suggesting that the FoxP3+ T cells inhibit the CTL response in vivo.

iii) FoxP3+ T cells inhibit the outgrowth of autologous leukemic T cells in ATLL.

iv) ATLL is not a tumour of regulatory T cells.

v) The site of integration of the HTLV-1 provirus in the T cell genome determines the size of each T cell clone in the host.
Molecular mechanisms of HPV-mediated TLR9 down regulation

Massimo TOMMASINO
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Cervical cancer development is linked to the persistent infection by high-risk mucosal human papillomaviruses (HPVs) types. The E6 and E7 major oncoproteins from this dsDNA virus play a key role in the deregulation of the cell cycle, apoptosis and adaptive immune surveillance. We have recently shown that HPV16, the most carcinogenic type among the high-risk subgroup, interferes with innate immunity by affecting the expression of Toll-like receptors (TLRs). Infection of human primary keratinocytes with HPV16 E6 and E7 recombinant retroviruses inhibits TLR9 transcription and hence functional loss of TLR9-regulated pathways. Similar findings were achieved in HPV16-positive cancer-derived cell lines and primary cervical cancers, demonstrating that this event occurs also in an in vivo context. Interestingly, E6 and E7 from the low-risk (LR) HPV6 are unable to down-regulate the TLR9 promoter. In addition, E6 and E7 from the HR HPV18, which are known to persist less competently in the host than HPV16, have reduced efficiency compared to HPV16 in inhibiting TLR9 transcription.

Initial data showed that the HPV16 E6 and E7 oncoproteins act through two distinct mechanisms in down-regulating TLR9 expression. We have now demonstrated that the HPV16 E7-induced TLR9 down-regulation is dependent on NF-κB pathway. Inhibition of the NF-κB pathway by different means, e.g. siRNA, dominant-negative mutant and chemical inhibitor, leads to the restoration of TLR9 expression in human primary keratinocytes transduced by HPV16 E7 retrovirus as well as in HPV16-positive cervical cancer-derived cell lines.

Interestingly, some of the cutaneous HPV types that appear to play a role in skin carcinogenesis have also the ability to down-regulate TLR9 expression.

This study reveals a novel mechanism used by HPV to suppress the host immune response by deregulating the TLR9 transcript, providing evidence that abolishing innate responses may be a crucial step involved in the carcinogenic events mediated by HPV.
Hepatitis C virus induced modification of hepatocyte metabolism and signalling: impact on viral persistence and tumor promotion

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Persistent hepatitis C virus (HCV) infection is a major global health problem. About 170 million people are chronically infected worldwide, which represents 2-3% of the world population. These patients are at high risk of developing steatosis, fibrosis and liver cirrhosis. Once cirrhosis is established, the rate of development of hepatocellular carcinoma (HCC) is 1 – 6 % per year.

Chronic inflammation induced by viral infection appears to be a major predisposing condition for liver cancer. In the case of hepatitis C, it is assumed that a persistently activated immune reaction targeting infected liver cells leads to increased cell proliferation and fibrogenesis, thus enhancing cirrhosis and HCC development. This indirect mechanism of tumor induction by HCV infection could explain why tumors most often develop only 10 – 30 years after primary infection and require additional etiological factors such as toxins or drugs (alcohol, aflatoxins, anabolic steroids), metabolic liver diseases, steatosis, non-alcoholic liver disease or diabetes.

Apart from these indirect mechanisms, there is increasing evidence that HCV itself, or specific viral proteins thereof, contribute directly to HCC formation. For instance, nonstructural protein 5B (NS5B) binds the retinoblastoma tumor-suppressor protein (Rb) and targets it for degradation. Moreover, core protein potently sequesters the RNA helicase DDX3 implicated in numerous processes including cell cycle control and innate immune signaling. Along the same line, the NS3/4A serine-type protease complex antagonizes the induction of Toll-like receptor 3 and retinoic acid induced receptor-I triggered type 1 interferon expression. These observations illustrate the complex interactions between HCV and the host cell and they probably have implications for the establishment of persistence and induction of HCC.
Session 7

Prevention of cancer
Neonatal Hepatitis B Immunization in Taiwan Results in Decrease of Hepatocellular Carcinoma in Childhood and Beyond

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Hepatocellular carcinoma (HCC) is a common cancer and is closely related to chronic viral infections of the liver, especially with the hepatitis B virus (HBV). In most Asian and African countries, 70%~80% of HCC are caused by chronic HBV infection that is contracted in early childhood. Perinatal mother-to-infant transmission plays a key role in establishing the chronic infection and, subsequently, possible eventual development of HCC. And thus, a large stride in preventing HCC relies on hepatitis B vaccination in infancy.

Neonatal immunization against HBV has been shown to decrease the prevalence of chronic HBV infection with an efficacy of ~85%. Most importantly, we have revealed a decrease of HCC in children in Taiwan ten years after universal neonatal HBV vaccination, which is the mainstay of the National Hepatitis B Vaccination Program launched in 1984. We continue monitoring the long-term trend of HCC in Taiwanese children and adolescents. Our most recent data showed that the incidence of HCC in 6~9-year-olds declined from 0.49 / 100,000 in those born before 1984 to 0.16 / 100,000 in those born after 1984 (p<0.001). For 10~14-year-olds, the incidence in those born before and after 1984 was 0.56 / 100,000 and 0.21 / 100,000, respectively (p<0.001). For 15~19-year-olds, it was 0.61 / 100,000 and 0.20 / 100,000, respectively (p<0.001). Those born after the Program but still got HCC were analyzed for the possible causes. Among the 23 children with HCC born after 1984, 22 were positive for hepatitis B surface antigen (HBsAg), again indicating the crucial role of HBV in causing their HCC. The chronic HBV infection in them was attributed to no vaccination in 10%, and vaccine failure in 90%. We conclude that prevention of HCC by hepatitis B vaccination has now extended from children to adolescents in Taiwan. Failure in preventing HCC was mainly caused by unsuccessful control of HBV infection by the highly infectious hepatitis B carrier mothers.
What's new with HCV vaccine?

Geneviève INCHAUSPE
Transgene - France

In spite of a sharp decrease in the incidence rate since the introduction of screening tests in the early 90’s, as well as the existence of a curative treatment in approximately 50% of cases, infections by HCV represent still a major burden worldwide (e.g., HCV is the leading cause of hepatocellular carcinomas in developed countries). The field of HCV vaccine development aims at either preventing or curing chronic infection. While programs aiming at developing a preventive vaccine were historically the first ones to be launched, results remain today limited, mainly obtained in animal models with the exception of a couple of trials conducted in healthy volunteers. In contrast, a larger number of players have entered the field of therapeutic vaccine development and have reached or are reaching phase II clinical development. This presentation will review major findings and discuss the challenges yet to be met as well as the novel concepts that have emerged in the last few years.
Next generation of HPV prophylactic vaccines

Lutz GISSMANN
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Due to the multiplicity of the high-risk HPV types related to cervical cancer the incidence of this disease can – even under optimal use of the currently available vaccines – only be reduced by 70-80%. The obvious option to reach a 100% protection is the addition of VLPs from other types that is currently being explored by the manufacturers. A promising approach to by-pass the immunologic identity of the individual HPV types is the use of the minor structural protein L2 that exists only in a few copies per virus particle. Immunization with L2 or with highly conserved immunogenic peptides derived thereof induces cross-neutralizing antibodies yet at the expense of relatively low titers. Future developments to improve the immune response include the use of very potent novel adjuvants or multimerization of the epitopes.

Another line of development is the generation of vaccines that combine prophylactic and therapeutic properties. The rationale behind this strategy is the fact that young women that had cleared their persistent infections (with or without clinical signs thereof) are still at risk for reinfection. A current development comprises chimeric VLPs that consist of L1 molecules fused to the non-structural protein E6 and/or E7.

Low-resource countries bear the highest burden of cervical cancer (more than 80% of the worldwide occurring cases). In the absence of any kind of Pap screening vaccination is the only realistic option to reduce the incidence of this disease. Obviously it is hard to imagine that the current vaccines will be widely used in such areas as they are by far too expensive and require refrigeration for storing. Therefore other options need to be explored, i.e. the production in cheaper expression systems such as E. coli or other suitable bacteria or recombinant plants. A further challenge, in particular for settings with low hygiene standards is the design of a vaccine suited for non-invasive («needleless») application such as direct by exposure to mucosal surfaces.
Chronic infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) infections are associated with the development of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) \(^1\). Major progress has been made in the treatment of chronic hepatitis B during the last decade \(^2\). Several antivirals were developed and approved including pegylated interferon alpha and nucleos(t)ide analogs (NUCs) such as lamivudine, adefovir, telbivudine, entecavir and tenofovir. Pegylated IFN administration may induce a decrease in viral load and an immune control of the disease associated with an improvement in liver disease and in some cases with a clearance of HBsAg from serum. The administration of NUCs leads to the control of both viral replication and liver disease activity \(^2\). Results of several studies suggest that both spontaneous and treatment-induced decrease of viral load may be associated with an improved clinical outcome (delayed progression to cirrhosis and to HCC) \(^3,4\). However, despite viral suppression can now be achieved in the majority of patients, viral cccDNA and integrated viral genomes cannot be cleared from the liver \(^5\). This may explain why HCC may still develop even after HBsAg seroconversion \(^6\). It was suggested that early treatment intervention may decrease the rate of viral genome integration\(^7\) and increase the rate of HBsAg clearance at younger age thereby delaying the risk of HCC development. Large cohort studies are still required to determine the impact of antiviral therapy on the prevention of HCC and its mechanism.

Chronic hepatitis C, by contrast to HBV infection, is a curable disease with the administration of a combination of pegylated IFN and ribavirin \(^8\). The development of cirrhosis is a major predisposing factor of HCC development in chronically HCV infected patients. Interestingly, it was shown that treatment induced HCV clearance is associated with a decreased risk of liver fibrosis progression and in some cases with a reversion of liver fibrosis/cirrhosis \(^9\). Several studies have shown a decreased risk of HCC in patients who had a sustained virologic response to IFN based therapy \(^10,11\). The direct antifibrotic and antioncogenic effect of IFN in virologic non responders remains a matter of debate. New antiviral strategies based on new immunotherapeutic approaches or specifically targeted antiviral therapy (protease, polymerase, NS5A, entry inhibitors) are under evaluation to increase the response rate to antiviral therapy in naïve patients and to provide new treatment options in previously non responder patients \(^12\).
References

Session 8

Therapy of virally induced cancers
Targeted therapy for virus induced cancers: the example of hepatocellular carcinoma

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Immunotherapy of hepatocellular carcinoma (HCC) is an attractive approach if one can specifically target tumor cells. A major difficulty is to identify and characterize “tumor associated” antigens that may be recognized by the host immune response to subsequently activate cytotoxic T-cells and promote tumor cell lysis. We have previously revealed that asparaginly-β-hydroxylase (AAH) is a highly conserved (99% identity when comparing the human to mouse sequence) protein expressed on the cell surface of >90% of HBV and HCV related human HCC. However, AAH is expressed at very low or negligible levels in normal adult tissues. This enzyme is involved in tumor metastasis by increasing cell motility, migration, and invasion through activation of Notch signaling. We investigated whether AAH immunization can induce antigen specific cellular immunity against expressing tumors. In this regard, dendritic cells (DCs) were employed as antigen-presenting cells to immunize mice against AAH. DCs were expanded in vitro by administration of FLT3L, purified from splenocytes and loaded with AAH coated magnetic microparticles; GFP was used as the control antigen. DCs were cultured for two days with cytokines that promote their maturation as measured by expression of cell surface markers. Mice were inoculated with a syngeneic HCC cell line and the tumor was allowed to grow (subcutaneously; s.c.) for one week followed by immunization (2X) over two weeks via s.c. injection of 5 x 10^6 DCs loaded with either AAH or GFP (control). We characterized DC phenotypes by measuring secretion of IL-12 and immunostaining for mature DC markers by FACS analysis. Induction of immunity against AAH was measured by INF-γ and IL-4 secretion from splenocytes derived from immunized mice. Cytotoxic T-cell activity was assessed using AAH expressing HCC target cells. DCs cultured for two days produced substantial amounts of IL-12, a cytokine essential for activation of naïve T-cells. Mature DC markers including CD40, CD54, CD80, CD86, and MHC class II were highly upregulated, and a CD8a-positive subset involved in tumor immunity was expanded as well. When mice were given two immunizations with AAH loaded DCs, lymphocytes from the immunized animals but not controls produced large amounts of INF-γ in response to AAH stimulation, whereas IL-4 production was minimally increased indicating the generation of T_h1-type immune response.
When lymphocytes isolated from AAH vaccinated animals were co-cultured with a murine AAH expressing HCC cell line, more than 40% were lysed compared to control. More important, immunization with the DC vaccine caused regression of established HCC tumors and prevented their subsequent growth in a murine model. Our observations suggest that immunization with AAH-loaded DCs induced an antigen-specific Th1-type immune response, lysed expressing HCC cells and prevented tumor growth in vivo. Therefore, AAH is a candidate antigen for DC vaccine induced immunotherapy of HBV and HCV related HCC.
Induction of objective clinical responses by immunotherapy with synthetic long peptides in patients with high grade HPV16-induced premalignant vulva lesions

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Therapeutic vaccination with a synthetic long peptide (SLP®) vaccine mediated the eradication of established human papilloma virus type 16 (HPV16)-positive tumors in mice and controlled wart growth and latent virus infection in rabbits persistently infected with cottontail rabbit papilloma virus. Subsequent phase I/II studies with an HPV16 SLP® vaccine, consisting of 13 long peptides covering the HPV16 E6 and E7 antigens, in patients with advanced HPV16-positive cervical cancer, revealed that this vaccine was safe and highly immunogenic. The purpose of the current study was to test the clinical efficacy of this HPV16 SLP® vaccine in HPV16-induced high grade vulvar intraepithelial neoplasia (VIN3), a premalignant epithelial disorder, spontaneous regression of which occurs in less than 2% of patients and in which recurrence after standard treatment is high.

In a phase 2 trial, 20 women with VIN3 were vaccinated three times sc in the limbs with a mix of the HPV16 E6 and E7 synthetic long peptides formulated in Montanide ISA-51. The endpoints were objective clinical responses, defined as reduction of at least 50% in lesion size (partial response) or complete regressions, and HPV16-specific T-cell responses, determined before and after vaccination. The vaccine was safe, as no side effects exceeding CTC grade 2 were observed. At 3 and 12 months after the last vaccination an objective response was observed in 12/20 (60%) and 15/19 (79%) patients respectively. Nine of them showed a complete and durable regression of the lesions at 12 months and at 24 months. The strength of the vaccine-induced HPV16-specific T-cell response was significantly higher in the group of patients with a complete regression of their lesions as compared to non-responders.

This study shows that in women with VIN3 objective clinical responses can be achieved by therapeutic vaccination with synthetic long peptides that is able to induce effective HPV16-specific T-cell responses.

Literature

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Viruses as tools: oncolytic paroviruses for the therapy of cancer

Jean ROMMELAERE
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Viruses belonging to the genus Parovirus, within the Paroviridae family, are able to replicate autonomously in the absence of helper viruses, in contrast to the adeno-associated virus members. The experimental infectivity and excellent tolerance of some rodent autonomous paroviruses in humans, together with their oncosuppressive effects in preclinical models, speak for the inclusion of these agents in the arsenal of oncolytic viruses under consideration for cancer therapy. In particular, wild-type parovirus H-1PV can achieve a complete cure of various tumors in animal models and kill tumor cells that resist conventional anticancer treatments. There is growing evidence that paroviral oncosuppression involves an immune component in addition to the direct viral oncolytic effect. This talk will summarize the recent assessment of H-1PV antineoplastic activity in glioma, pancreatic ductal adenocarcinoma, and non-Hodgkin lymphoma models, laying the foundation for the present launch of a first phase I/IIa clinical trial on glioma patients.
Final session
Why do viruses cause tumors? New technologies to human tumor virology

Patrick S. MOORE
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Viral cancers comprise ~10-15% of human cancers world-wide. To identify new human tumor viruses, our laboratory (jointly led by Yuan Chang and Patrick Moore) developed digital transcriptome subtraction (DTS) to analyze high-throughput cDNA sequencing data. We applied DTS to four Merkel cell carcinoma (MCC) tumors and identified two novel transcripts belonging to a previously undescribed human polyomavirus, Merkel cell polyomavirus (MCV). Overall, MCV mRNA was detected at ~10 transcripts per million, or approximately 5 transcripts per cell on average. Approximately 80% of MCC tumors harbor MCV as an integrated, clonal genome and express the viral large T oncoprotein. Tumor-derived T antigens, however, have secondary mutations that render the virus noninfectious. These mutations prevent from DNA replication from the integrated adventitious viral origin that would otherwise generate lytic virus. Although MCC is rare, antibody tests demonstrate that MCV infection is ubiquitous among healthy adults. It is likely that accumulation of independent mutational events (genome integration and T antigen truncation) is required for this virus to initiate tumor cell transformation.
Posters session
Targeting the enzymes of nucleoside biosynthesis provides a way for discovery of novel ‘dual specificity’ inhibitors of hepatitis C virus

Larissa BALAKIREVA
NovoCIB - France

The traditional single-drug, single-target paradigm in drug discovery is not always the most successful and there are numerous examples of drugs that mediate their effects through multiple targets. Ribavirin, a broad-spectrum antiviral nucleoside analogue used to treat hepatitis C (HCV) infection, is a classic example of such a drug. A number of possible mechanisms have been proposed to account for the antiviral activity of ribavirine – its monophosphorylated form inhibits cellular Inosine-Monophosphate Dehydrogenase (IMPDH), whereas tri-phosphorylated form, after being incorporated by viral RNA-dependent RNA-polymerases, induces “error catastrophe” and inhibits RNA synthesis.

Numerous nucleoside analogues (NA) are currently used to treat HBV, HIV and herpes simplex viral infections. They are usually designed to inhibit one viral target, the polymerase. Dual inhibition of viral polymerase and cellular nucleotide biosynthesis by the same NA could result in its higher efficiency.

For rapid evaluation of monophosphate forms of NA as IMPDH inhibitors, we have developed an in vitro enzymatic assay consisting of cloned human nucleoside kinases and cloned human IMPDH type 2. In this assay the monophosphorylation of nucleoside analogue is provided by specific nucleoside kinases, and enzymatically produced NA-monophosphate is simultaneously tested for IMPDH inhibition. This combined test has been validated with nucleoside analogues ribavirin and mizoribine, both known inhibitors of human IMPDH.
Small protein-mediated inhibition of frizzled-7 displays antitumor properties in hepatocellular carcinoma

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INSERM - France

Background: Inhibition of the Frizzled-7 receptor (FZD7) by compounds antagonizing its binding to Dishevelled (DVL) was recently shown as exerting anticancer properties in nonliver cancer cells. We previously reported the common expression of FZD7 in virus and non-virus-related-hepatocellular carcinoma (HCC) and its role for controlling the cancer phenotype.

Objectives: Our aim was to design a strategy to inhibit FZD7-mediated signalling and to assess the potential antitumor effect on HCC cells.

Methods: We have designed small interfering proteins “RHPDs” able to enter hepatocytic cells and competitively antagonizing the binding of FZD7 to the PDZ domain of DVL. RHPDs were tested on a panel of human HCC cell lines for apoptosis (annexin-V staining) and cell viability (MTT assay). The FZD7-related downstream beta-catenin, PKC, JNK pathways were explored (TOPFlash assay, Western Blot, semi quantitative RT-PCR). The in vivo antitumor effect of RHPDs was test on the SV40-Tag transgenic mouse lineage.

Results: We have shown that RHPDs enter within hepatocytic cells, leading to apoptosis depending of their affinity for PDZ, with therapeutic index between cancerous cells and primary hepatocytes. RHPD-mediated apoptosis was linked to beta-catenin inhibition as well as PKC-delta activation in a TP53-independent manner. From human tissues, FZD7-positive HCCs showed PKC-delta repression, and experimental prolonged ectopic activation of FZD7 (lentivirus delivery of WNT3 and FZD7) in liver progenitor cells led to PKC-delta repression. Intra-tumor injection of RHPDs slowed down in vivo HCC progression in transgenic mice.
Analysis of infectivity of hepatitis B virus (HBV) mutants resistant to nucleoside analogs

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Background and aims: Nucleoside analogue therapies for chronic HBV infection may lead to the emergence and selection of HBV polymerase (pol) variants which may also harbor mutations in the small envelope protein (S-HBs). To understand their mechanism of selection, we analyzed the in vitro secretion capacity and infectivity of the main viral mutants resistant to lamivudine (LAM) and adefovir (ADV).

Methods: HBV-resistant mutants generated by site-directed mutagenesis of a genotype A adw2 wild type (WT) construct were introduced by transfection into HepG2 cells as well as WT construct. Cell clones were selected under G418 treatment and the following permanently expressed mutants were studied: rtL180M+rtM204V, rtV173L+rtL180M+rtM204V, rtM204I, rtL180M+rtM204I, rtN236T, rtA181V, rtA181V+rtN236T, rtA181T, and rtA181T+rtN236T. The expression and secretion of HBsAg or viral particles were analyzed by immunological assays and electron microscopy. The infectivity of the different HBV mutants was studied in HepaRG cells that are susceptible to wild type HBV, by quantification of viral mRNA by qRT-PCR and immunological analysis of HBV surface antigens (HBsAg).

Results: Only mutants harboring the rtA181T LAM/ADV resistance mutation were unable to synthesize HBsAg or secrete Dane particles, due to a stop codon introduced in the S domain of the envelope gene which overlaps with the pol gene (sW172stop). All resistant mutants were able to secrete HBeAg at different rates. Except for rtA181T mutants, all other resistant mutants could infect HepaRG cells, as determined by HBsAg and HBV RNA detection and quantification in infected cells 12 days after inoculation, by immunological labelling and qRT-PCR, respectively. The rtM204I mutant, which was a low replicator in the producer HepG2 cell line, was weakly infectious compared to rtN236T mutant, which was almost as infectious as WT HBV in our experimental conditions.

Conclusions: Our in vitro results suggest that infectivity of HBV polymerase mutants that are resistant to antivirals is modulated by mutations in the overlapping surface gene. These results provide new insight into the capacity of the resistant mutants to spread and emerge in the liver of treated patients, and highlight the possibility of resistant mutant transmission in the general population.
Worldwide distribution of human papillomavirus 58 variant lineages

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Introduction: The disease impact associated with non-vaccine covered human papillomavirus (HPV) types shows a considerable geographical variation. Cervical cancers from East Asian populations showed a much higher prevalence of a worldwide rare type (HPV58), including Shanghai (26%), Taiwan (18%), Korea (16%), Hong Kong (10%) and Japan (8%). Previous studies suggested that host genetic factors and circulation of high-risk variants in East Asia could play a role. To date, information on the sequence variability of HPV58 is scarcely available. This study established a phylogenetic classification system for HPV58 variants to facilitate further studies.

Materials and Methods: 401 HPV58-positive specimens collected from 15 cities/countries were sequenced for the whole length of E6, E7, E2, E5, L1 and LCR regions. Maximum likelihood trees and Bayesian trees were constructed to examine the phylogenetic relationship.

Results: Altogether, 268 unique concatenated E6-E7-E2-E5-L1-LCR nucleotide sequences of HPV58 variants were assembled. The maximum-likelihood and the Bayesian algorithms showed a similar tree topology that revealed four distinct clusters, designed according to their overall prevalence as lineage A, B, C, and D. The L1 ORF was the most representative region for phylogenetic grouping. Lineage A was the most prevalent variant lineage in each of the four regions studied, and it dominated in Europe (88.2%) and America (72.6%). Lineage B was the second most prevalent variant found in Asia (27.3%) and America (17.5%), but uncommon in Europe (6.5%), and rare in Africa (0%). Lineage C showed a predilection for Africa and was the second most prevalent variant found (40.6%). Lineage D accounted for 11.8% of Asian isolates, but uncommon in America (7.9%) and Africa (2.9%), and was rare in Europe (0%). Overall Asia and America had a co-circulation of a wider spectrum of HPV58 variants of different lineages; whereas Africa was predominated by lineages A and C, and Europe was mainly restricted to lineage A.

Conclusions: HPV58 variants can be grouped into four lineages. Further study on the epidemiological and risk implication of this lineage classification system is warrant.
Interaction between HBx and E4F: a possible mechanism of HBV-induced hepatocarcinogenesis

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Double Hybrid experiments have shown that the hepatitis B virus protein HBx could interact with the transcription regulatory protein p120 E4F. In vitro, this interaction led to the repression of p120 E4F-regulated gene transcription. However, the existence and functional impact of this interaction has never been demonstrated in vivo (Rui et al. 2006).

P120E4F is a key player in the control of mammalian embryonic and somatic cell proliferation and survival. Mouse embryos lacking E4F die at an early developmental stage, whereas enforced expression of p120E4F in various cell lines inhibits cell cycle progression. The anti-proliferative of p120E4F effects have been shown to depend on its capacity to repress transcription and to interact with p53. Aside from its effect as transcription factor, p120E4F has been shown to exert an unconventional ubiquitin-ligase activity towards p53, and there is evidence that some of its growth-suppressive effects are mediated through p53 (LeCam et al. 2006).

In this study, we have analyzed the expression of p120E4F in various cell lines derives from hepatocellular carcinoma and we have investigated its capacity to form complexes with HBx. We found that p120E4F is constitutively expresses in the nucleus of HCC cells independently of their p53 or HBx expression status. However, in HepG2 cells, transfection and overexpression of HBx resulted in an increase in p120E4F levels. To further investigate the interactions between the two proteins, we have performed cell fractionation experiments to determine their sub-cellular localization, as well as co-immunoprecipitation studies.

HBx is an important protein in HBV-mediated liver carcinogenesis, due to its capacity to interact with multiple cellular partners involved in the control of proliferation and of genetic stability. Further functional studies will be required to determine whether targeting p120E4F is one of the mechanisms by which HBx may affect cell transformation.

Human T-cell Leukemia Virus Type 2 produces a spliced antisense transcript encoding a protein that lacks a bZIP domain but inhibits Tax2-mediated transcription

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Human T-cell Leukemia virus type 1 and type 2 retroviruses infect T-lymphocytes. While HTLV-1 infection causes leukemia, HTLV-2 has not been demonstrated to be the agent of a hematological malignant disease. The minus strand of the HTLV-1 genome encodes HBZ, a protein which could play a role in the development of leukemia in infected patients.

Herein, we demonstrate that the complementary strand of the HTLV-2 genome also encodes a protein that we named APH-2 (Antisense Protein of HTLV-2). APH-2 mRNA is spliced, polyadenylated and initiates in the 3’LTR at different positions. This transcript was detected in different tested HTLV-2-infected cell lines as well as in one HTLV-2 carrier.

The APH-2 protein is 183 amino acid long and is localized in the nucleus of transfected cells. Despite the lack of a bZIP domain consensus sequence, APH-2 represses Tax2-mediated transcription. Our results demonstrate the existence of an antisense strand-encoded protein in HTLV-2, which could represent an important player in the development of disorders such as the lymphocytosis that is frequently observed in HTLV-2 patients.
Frequent, numerous, early, and virus-specific telosome dysfunctions during hepatocarcinogenesis

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Hepatitis viruses cause hepatocellular carcinoma (HCC), one of the most common cancers worldwide. HCC is a multistep disease that includes well-individualized histological stages such as chronic hepatitis, cirrhosis and the bona fide malignant tumor stage. Among the numerous genetic defects that rely with hepatocarcinogenesis, telomere abnormalities seem to play a role both in tumor promotion and maintenance. Telomeres are the chromosome extremities that are protected by specific proteins, the shelterin complex and by additional factors also implicated in other cell functions. This set of proteins, called the telosome, works in concert with the telomerase, a ribonucleoproteic complex that catalyzes telomere elongation. Telosome and telomerase abnormalities are the hallmark of many cancers and might possess diagnostic, prognostic and therapeutic implications in HCC. Recent studies have investigated hTERT - the gene encoding the catalytic telomerase activity, and certain telosome factors in HCC. They evidenced significant modifications in the expression pattern of these factors in tumor and/or pretumoral cells whereas conflicting results have been published with regard to the levels hTERT expression during hepatocarcinogenesis. Here we hypothesized that telosome dysregulation might depends i. on the cause of HCC, i.e. might be different between HCV versus HBV-induced tumor; ii. on the stage of the disease, i.e. might be different between normal versus cirrhotic versus cancerous liver. For the present study we investigated these hypotheses at the transcriptional level. The materials corresponded to 80 liver biopsies deriving fro 40 patients with HCC, obtained after informed consent and including 10 HBV-, 10 HCV-, 10 alcohol-related, and 10 idiopathic HCC. For each of the 3 former categories, tumor and cirrhotic peritumoral tissues were obtained while for the latter, peritumoral tissue was histologically normal and served as control. The method consisted in real time PCR amplification of the main telosome genes and the main known active and inactive hTERT isoforms.

These genes included 5 hTERT isoforms, hTR, shelterin protein genes (TRF1, TRF2, POT1, TIN2, PTO, and RAP1) and a set of multifunctional genes that encode proteins belonging to the telosome (hMRE11a, hMRE11b, RAD50, Ku70, Ku80, TANK1, TANK2, DNA-PKcs, and NBS1) or having a known role in controlling telomerase activity (AKT1/2, PTEN, cMYC, and MAZ).
Comparison were made with the Mann Whitney test and p<0.05 defined statistically significant differences. Firstly we compared cirrhotic and normal liver samples. For HBV, the following genes were significantly underexpressed in the cirrhotic tissue: hTERT (active +A+B isoform), hTR, cMyc, PTEN, MAZ, AKT1, AKT2, POT1, PTOP, RAP1, TIN2, TRF1, TRF2, DNA PKcs, hMRE11 A, hMRE11 B, KU70, KU80, NBS1, PINX1, TANK1, TANK2. There was not significant difference in the expression of the remaining 4 genes. For HCV, POT1, and RAP1 were overexpressed in cirrhosis without evidence for significant transcriptional change for the remaining 24 genes. For alcohol-related HCC the +A+BhTERT isoform was underexpressed in the cirrhotic tissue while cMyc, AKT1, AKT2, POT1, RAP1, TIN2, TRF1, DNA PKcs, hMRE11a, hMRE11b, Ku70, Ku80, PINX1, RAD50, and TANK1 were overexpressed. Secondly we compared gene expression between HCC and cirrhosis for HBV, HCV, and alcohol-related HCC. For these 3 tumor types, +A+BhTERT overexpression in the cancerous tissue was the unique transcriptional modification. These results validate our hypotheses and show that virus-associated HCC add-up numerous telosome dysfunctions that appear to be virus-specific and that occur early during the course of the disease. Time-dependent modulation of hTERT expression is remarkable as it demonstrate a precancerous hTERT transcriptional repression known to favor, in a context of impaired p53 function, to promote chromosomal instability. Finally, monitoring telosome dysfunctions at the cirrhotic stage might help early diagnose cirrhosis or HCC in exposed individuals while targeting these dysfunctions might help prevent HCC in the same population.
Characterization of the molecular mechanism of Epstein-Barr virus-mediated inhibition of the innate sensor TLR9

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Epstein-Barr Virus (EBV) infects most of the human population and is associated with a number of human diseases including cancers. Evasion of the immune system and chronic infection is an essential step for EBV-associated diseases. In this study we show that EBV can alter the regulation and expression of Toll-Like Receptors (TLRs), the key effector molecules of the innate immune response. EBV infection of human primary B cells resulted in the inhibition of TLR9 functionality. Stimulation of TLR9 on primary B cells leads to the production of IL-6, TNFα and IgG, which is inhibited in cells infected with EBV. We observed that EBV exerts its inhibitory function by decreasing TLR9 mRNA and protein levels. In addition, we determined that the major EBV oncoprotein, LMP1, plays a key role in the inhibition of TLR9 transcription. TLR9 promoter activity as well as mRNA and protein levels were strongly reduced in a B cells transiently transfected with LMP1 expression construct or transduced with LMP1 recombinant retrovirus. LMP1 induces, TLR9 via the activation of the NF-κB pathway. LMP1 mutants deficient in activating the NF-κB pathway inversely restored TLR9 transcription. Accordingly, inhibition of NF-κB pathway in LMP1-expressing cells resulted in TLR9 promoter activity. Together, our study reveals a novel mechanism used by EBV to suppress the host immune response by deregulating the TLR9 transcription through LMP1-mediated NF-κB activation.
Transcriptional and translational analyses of the cell responses induced by the integration of a foreign DNA into their genomes

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In numerous situations, cells undergo the incorporation of additional genetic materials into their genomes. For example, the replication cycle of retroviruses requires the integration of the viral genome into the genomic DNA of the infected cells. Adeno-Associated Viruses (AAV) may also incorporate their genomes into that of the host cells. The aim of our work is to identify cell signalling pathways developed in response to the integration of additional DNA. We ask the following questions: does viral integration provoke important perturbations into the cells or, on the contrary, do the cells passively undergo this integration? If such a cellular response exists, which factors are involved? What are their roles in the cells?

We experimentally performed the integration of additional DNA into human primary cell genomes by using a Human Immunodeficiency Virus (HIV)-based lentiviral vector. We also used a control vector (the same vector that was rendered unable to perform the integration process). This control vector was used to detect and eliminate the cell responses due to viral steps other than the integration one (entry, reverse transcription, transport…). By PCR, we detected the infection of the target cells with each vector. Then, by using a technique of nested quantitative PCR, we revealed the integration of the integrative vector but not that of the control vector. We further studied the integration of the integrative vector at different times after infection. This kinetic of integration allowed us to define the time post-infection the most relevant to our experiments. We are now analyzing the transcriptome (whole RNAs population) and the proteome (whole proteins population) of the cells infected with either the integrative vector or the control one. Our analyses focus on RNAs and proteins whose expression is specifically up- or down-regulated following the integration (i.e. up- or down- regulated following the infection with the integrative vector but not following the infection with the control one).

As the integration of additional DNA not only concerns viral processes but also cellular processes (transposition of mobile elements, chromosomal reorganizations during cancers induction…), data obtained in this study will allow (i) the development of defensive strategies against integrative viruses (such as HIV) as well as against diseases characterized by an injury of the genome integrity and (ii) the evaluation of cellular perturbations caused by the integration of a therapeutic vector into the cell genomes during gene therapy or gene transfer experiments.
Effects of the TP53 p.R249S mutant on proliferation and apoptosis in human hepatocellular carcinoma cell lines: interaction with Hepatitis B Virus X protein

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Background/Aim
Hepatocellular carcinoma (HCC) is frequent in West Africa and South-East Asia, where it is a leading cause of cancer-related death. The major risk factors are hepatitis B virus infections and aflatoxin B1 exposure. Aflatoxin B1 metabolites bind to several positions in TP53 gene and generate mutations. The hotspot mutation R249S (AGG to AGT, arginine to serine) accounts for more than 50% of TP53 mutations in aflatoxin B1-related HCC. This specificity suggests that p.R249S may confer a selective advantage during hepatocarcinogenesis. We have investigated the biochemical and biological properties of p.R249S in HCC cell lines.

Methods/Results
We shown that p.R249S has lost the capacity to bind to p53 response elements and to transactivate p53 target genes. In p53-null Hep3B cells, stable transfection of p.R249S or of another mutant, p.R248Q, did not induce significant changes in cell proliferation and survival after cytotoxic stress. In contrast, in PLC/PRF/5 cells that constitutively express both p.R249S and the HBV antigen HBx, the two proteins form a stable complex implicated in proliferation. Silencing of either p.R249S or HBx with siRNAs slowed down proliferation, with no additive effects when both factors were silenced. This observation suggests that the two proteins act within the same pathway. In human HCC samples, mutation at codon 249 did not correlate with p.R249S protein accumulation, suggesting that the maintenance of p.R249S is not essential for tumour progression.

Conclusion
We suggest that p.R249S contributes to hepatocarcinogenesis through stable interaction with HBx and that this mechanism is of particular relevance at early steps of carcinogenesis.
The NACos protein, ubinuclein, negatively regulates the Epstein-Barr Virus productive cycle in epithelial cells.

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The Epstein-Barr Virus (EBV) productive cycle is initiated by the expression of the viral trans-activator EB1, which belongs to the basic-leucine zipper transcription factor family. We have previously identified the cellular NACos (Nuclear and Adherent junction Complex components) protein ubinuclein (Ubn-1) as a partner for EB1, but the function of this complex has not been studied. Here, we have evaluated the consequences of this interaction on the EBV productive cycle and find that Ubn-1 over-expression represses the EBV productive cycle whereas, Ubn-1 down regulation by shRNA, increases virus production. By ChIP assay, we show that Ubn-1 blocks EB1-DNA interaction. We also show that in epithelial cells, re-localization and sequestration of Ubn-1 to the tight junctions of non-dividing cells allows a better activation of the productive cycle. We propose a model in which Ubn-1 is a modulator of the EBV productive cycle: in proliferating epithelial cells, Ubn-1 is nuclear and inhibits activation of the productive cycle whereas in differentiated cells, Ubn-1 is sequestrated to tight-junctions, thereby allowing EB1 to function in the nucleus.
Optineurin cooperates with TAX1BP1 to potentiate ubiquitin-dependent activation of NF-κB by Human T-cell Leukemia Virus type 1 Tax protein.

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NF-κB controls the expression of key proteins involved in cell survival. Contrasting with the normal situation where the NF-κB pathway is only transiently activated, NF-κB is constitutively activated in human cancers such as B-cell chronic lymphocytic leukemia and lymphomas. The elucidation of the mechanisms underlying this constitutive activation is a scientific challenge, which has strong therapeutic potential. Novel roles of polyubiquitin chains in the activation of NF-κB were recently highlighted, suggesting a crucial function for this post-translational modification in the NF-κB signaling pathway. In Human T-cell Leukemia Virus (HTLV)-1-triggered cell transformation, constitutive activation of NF-κB by the virally-encoded Tax protein plays a central role. Interaction of polyubiquitinated Tax with the regulatory component of IκB kinase, IKK-γ/NEMO has been shown to be necessary for Tax-induced NF-κB activation. However, little is known about how polyubiquitination of Tax is modulated.

We recently identified optineurin, a cellular protein that shares sequence similarity with NEMO, as a potential interactor of Tax. Known cellular functions associated with optineurin encompass architecture of the Golgi, regulation of the secretory pathway, and inhibition of TNF-α signaling. Using co-immunoprecipitation assays and immunofluorescence studies, we demonstrate the interaction between Tax and optineurin in Tax-transfected cells as well as HT-LV-infected cell lines. Furthermore, we demonstrate that similarly to NEMO, Tax interaction with optineurin is dependent upon Tax ubiquitination. Interestingly, overexpression of optineurin leads to a dose-dependant increase of Tax-induced NF-κB activation. We also show that interaction with optineurin stabilizes Tax1 ubiquitinated forms. To further understand the mechanism underlying the modulating effect of optineurin, we investigate the involvement of TAX1BP1, a cellular protein known to interact both with Tax and with the deubiquitination machinery. We show that Tax, optineurin and TAX1BP1 form a complex, and that TAX1BP1 cooperates with optineurin for the modulation of Tax function.

Altogether, our results bring new insights into the regulatory mechanisms of Tax ubiquitination in regards to Tax activity on NF-κB, and indicate that optineurin could be a major modulator of Tax activity.
Hepatitis B virus induces expression of antioxidant response element (ARE)-regulated genes by activation of Nrf2

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Background: The expression of a variety of cytoprotective genes is regulated by short cis-acting elements in their promoters, called antioxidant response elements (AREs). A central regulator of ARE-mediated gene expression is the NF-E2 related factor 2 (Nrf2). Nrf2/ARE-regulated genes are crucial for the maintenance of cellular integrity. Moreover, integrity of Nrf2 was shown to be crucial for liver regeneration.

Methods: Reporter gene assays, western blot analyses and activity assays were performed to study the effect of HBV on the expression of ARE-regulated genes. To study the relevance of Nrf2 for HBV replication primary mouse hepatocytes isolated from Nrf2-deficient mice were prepared.

Results: Human hepatitis B virus (HBV) induces a strong activation of ARE-regulated genes in vitro and in vivo that depends on the functionality of Nrf2. The HBV-regulatory proteins trigger via c-Raf and MEK the induction of Nrf2/ARE-regulated genes. Radical formation is not relevant for the HBV-dependent induction of Nrf2/ARE-regulated genes. However, the Nrf2/ARE-mediated induction of cytoprotective genes by HBV results in a better protection of HBV positive cells against oxidative damage as compared to control cells. Furthermore, there is a significantly increased expression of the Nrf2/ARE-regulated proteasomal subunit PSMB5 in HBV positive cells that is associated with a decreased level of the immunoproteasome subunit PSMB5i. In accordance with this finding, HBV positive cells display a higher constitutive proteasome activity and a decreased activity of the immunoproteasome as compared to the control cells.

In conclusion, these data suggest that HBV triggers the expression of Nrf2/ARE-regulated genes to ensure the survival of the infected cell and thereby promote the establishment of the infection. The HBV-dependent induction of cytoprotective genes might be relevant for the design of therapeutic strategies to affect HBV-associated HCC.
The expression of a variety of cytoprotective genes is controlled by a cis-acting sequence, the antioxidative response element (ARE). The transcription factor Nrf2 plays a crucial role in the control of ARE-dependent genes. Recently, it was shown that integrity of Nrf2 is required for liver regeneration.

Reporter gene assays and western blot analyses revealed that Hepatitis C virus (HCV) impairs the expression of ARE—regulated genes. Moreover, HCV inhibits the induction of ARE-dependent genes by electrophilic reagents. Overexpression of constitutive active Nrf2 does not compensate the inhibitory effect of HCV on ARE-regulated genes, indicating that a change in the amount of Nrf2 is not causative for the inhibitory effect of HCV. Small Maf proteins heterodimerize with Nrf2 and thereby affect Nrf2 activity. Overexpression of small Mafs inhibits Nrf2. HCV increases the level of small Mafs. This was confirmed by IHC of liver samples derived from HCV-positive patients. Confocal immunofluorescence microscopy shows that in HCV replicating cells in addition to the increased amount the subcellular localization of small Mafs is affected. In control cells small Mafs are localized in the nucleus, in HCV replicating cells small Mafs are found in the cytoplasm colocalized with the replicon complex. A more detailed analysis suggests that HCV core protein in the context of HCV replication induces the delocalization of sMafs and the resulting impairment of Nrf2/ARE induction by HCV.

The HCV-dependent inhibition of Nrf2/ARE-dependent gene expression results increased levels of intracellular radicals as evidenced by elevated levels of oxidatively damaged proteins and DNA. Taken together these data demonstrate conclude that HCV impairs the activation of Nrf2/ARE- regulated genes by increasing the amount of small Mafs and their delocalization into the cytoplasm. The decreased expression of Nrf2/ARE regulated genes that are crucial for a variety of cytoprotective processes and liver regeneration might play a role for HCV-associated pathogenesis.
HTLV-1 neutralizes Menin repression on telomerase activity

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HTLV-1 (Human T-cell Leukemia virus, type 1) is a retrovirus responsible for adult T-cell leukemia (ATL) in 5% of infected people. ATL is characterised by a monoclonal proliferation of infected CD4+ T lymphocytes in which a high telomerase activity associated to a poor prognosis has been observed. Telomerase activity in cancer cells depends on the transcription of the \textit{hTERT} catalytic subunit gene. We have shown that the HBZ (HTLV-1 bZip factor) retroviral protein together with JunD stimulates the \textit{hTERT} proximal promoter.

As Menin, a cellular partner of JunD, negatively regulates telomerase, we analyzed the effects of Menin and HBZ on the activation of the \textit{hTERT} promoter. Our results underline that HBZ and Menin exert opposite effects on the transcriptional activity of JunD and demonstrates that HBZ is able to relieve the Menin-mediated suppression of telomerase. Such an antagonism is dependent on the recruitment of Histone Deacetylases (HDACs) and p300 by Menin and HBZ, respectively. Indeed, Trichostatin A, an HDAC inhibitor that reverses the Menin-mediated suppression of JunD transcriptional activity facilitates the effect of HBZ on the \textit{hTERT} promoter. Functional and chromatin immunoprecipitation assays demonstrate the specific recruitment of p300 by HBZ on the \textit{hTERT} promoter.

Finally, RT-qPCR assays show that primary ATL cells express high levels of HBZ and \textit{hTERT} as well as of MEN-1 transcripts, supporting that HBZ is critically involved in the stimulation of \textit{hTERT} transcription by repressing the inhibitory activity of Menin.

These results reveal that HBZ is acting as a tumor promoting protein and emphasize its critical role during HTLV-1-induced leukemogenesis.
Epigenetic changes induced by HBV infection and other major risk factors in hepatocellular carcinoma

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Hepatitis B virus (HBV) infection is a major public health problem worldwide and one of the most important risk factor for developing liver disease including hepatocellular carcinoma (HCC). While viral detection protocol has been improved, the mechanisms by which HBV triggers hepatocarcinogenesis remain poorly understood. It is now well known that aberrant epigenetic events are common in malignant cells and that interplay between genetic and epigenetic alterations contribute to most human cancers including HCC. In the present project, we aim to study the epigenetic changes associated to HBV infection in HCC samples. To this end, we have studied the global DNA methylation changes and the miRNA expression in HCC associated with HBV infection or other known risk factors (HCV infection and alcohol consumption). Using high-throughput epigenomic tools (DNA methylation arrays), we identified specific DNA methylation signatures in HCC tumors and matched surrounding (cirrhotic) tissues. We also found a small panel of genes exhibiting a specific DNA methylation pattern according to risk factors exposures, especially with HBV infection. Our genome-wide miRNA analysis, using micro arrays platforms, revealed that their expression is strongly influenced by risk factors exposure, reinforcing the role of epigenetic mechanisms as an integrative platform for environmental signals. Together, these results highlight an essential role for epigenetic changes in the mechanism of HBV-mediated HCC and may lead to new important information for the development of novel strategies for epigenetics-based diagnostics and treatment of hepatocellular carcinoma.
Interactions between Hepatitis B Virus and Aflatoxin B1 in the HepaRG cell model: effects on viral replication, adduct formation and repair, and p53 response.

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Background and aims: In sub-Saharan Africa and South-East Asia, endemic chronic infection by hepatitis B virus (HBV) and dietary exposure to aflatoxin B1 (AFB1) play a synergic role in the development of chronic liver disease and Hepatocellular Carcinoma (HCC). AFB1 is produced by Aspergillus flavus that contaminate foodstuffs in hot and humid areas and is bioactivated in the liver into an epoxide which leads to DNA adduct formation and mutagenesis. A specific mutation in the tumor suppressor gene TP53 is often generated in this context (AGG to AGT at codon 249, R249S). However, the molecular mechanism of the synergistic effect of HBV and AFB1 are still largely unknown. We have taken advantage of the unique features of a novel cell line, HepaRG, to investigate the interplay between exposure to AFB1 and infection by HBV. HepaRG can undergo differentiation in vitro into hepatocyte-like cells that can be infected by HBV, thus providing a unique model to examine the effects of both factors in a culture system. Methods: HepaRG cells were infected by HBV (genotype D), then exposed to different doses of AFB1 (0-5μM) for 4 hours and maintained for up to 48hrs. Viral replication was analyzed by detecting HB surface Antigen (HBsAg) and viral DNA in the medium by ELISA and quantitative (q) PCR, respectively. Viral DNA and RNA were also monitored in cellular extracts. Adduct formation and persistence were examined by immunodot blot assay using an antibody against AFB1-DNA adducts. Induction of p53 and of its target genes p21WAF1 and HDM2 were assessed by Western Blot and RT-qPCR.

Results: Exposure to AFB1 decreased secretion of HBsAg in the medium but not extracellular viral load. HBV transcription and viral DNA levels were significantly decreased after 48hrs of treatment with 5μM AFB1. Adduct formation was dose-dependent and short-lived, with a peak at 4hrs after exposure to AFB1 followed by return to basal levels after 20hrs. There was a tendency for less adduct formation or persistence in cells infected with HBV. Induction of p53 and activation of target genes in response to AFB1 was essentially similar in both infected and non-infected HepaRG.

Conclusions: Exposure to AFB1 down-regulates several parameters of HBV replication. In contrast, HBV infection does not profoundly modify adduct formation and subsequent p53 induction in response to AFB1. Further studies are required to determine whether infection by HBV specifically enhances AFB1 adduct formation leading to mutagenesis at codon 249 in TP53 gene.
High expression of the anti-apoptotic Bfl-1 and Bcl-x<sub>L</sub> proteins contributes to resistance to apoptosis of HTLV-1 transformed T-cells

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Elevated expression of Bcl-2, Bcl-x<sub>L</sub> and Bfl-1 anti-apoptotic members of the Bcl-2 family was previously reported in several cancers. In B cell malignancies, overexpression of these proteins contribute to apoptosis-resistant phenotype and chemoresistance. However, few data concerning viro-induced cancers are available, especially for the human T-cell leukemia virus type 1 (HTLV-1). Infection by HTLV-1 may lead to T-cell transformation and to adult T-cell leukemia/lymphoma (ATLL). This process requires Tax1, which up-regulates Bcl-xL expression by activating the NF-κB pathway. As the expression of Bfl-1 is also induced by the NF-κB pathway, we hypothesized that Bfl-1, in addition to Bcl-xL, may be involved in resistance to apoptosis during T-cell transformation.

Our results showed that Bfl-1 expression, both at messenger and protein levels, is restricted to HTLV status in transformed T-cell lines, while Bcl-2 and Bcl-xL are overexpressed in those cells compared to HTLV negative T-cell lines. To confirm theses data, we now search for bfl-1, bcl-xL and bcl-2 mRNA levels in ATLL cells from patients of different disease subtypes. Interestingly, after ectopic expression of Tax in HeLa cells, we observed an induction of bfl-1 mRNA level of about 80-100 fold by real-time RT-PCR and only an increase of bcl-xL mRNA level of about 2 fold compared to empty vector. As previously described, Tax was unable to modulate bcl-2 expression, suggesting that other viral protein(s) may be involved. Finally, using short hairpin RNA strategy, we demonstrated that HTLV transformed T-cell lines are differentially sensitive to apoptosis depending on the targeted anti-apoptotic member. By flow cytometry, we showed that bfl-1 and/or bcl-xL silencing partly restored cell death of HTLV-1-transformed T-cell lines, whereas bcl-2 and bcl-xL silencing in HTLV-2-transformed T-cell lines partly induced their spontaneous cell death.

Our data demonstrate for the first time that Bfl-1, as well as Bcl-xL, is an essential protein for survival of HTLV-1 transformed T-cells and suggest that both Bfl-1 and Bcl-xL might be therapeutic targets in HTLV-1-induced ATLL. To date, small molecules (ie: ABT-737) targeting Bcl-xL and Bcl-2 are available, but no for Bfl-1. In this context, we develop a strategy in order to identify small molecules blocking Bfl-1 anti-apoptotic activity and then the potential leads will be tested in in vitro and in vivo HTLV-1 models.
Glucocorticoid-induced reactivation of an occult HBV infection in a patient co-infected with HIV

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About 20% of HIV+ patients are co-infected by HBV due to the same routes of transmission. With the advent of HAART to control HIV, HBV infection is one of the major causes of mortality in HIV+ patients. Occult HBV infections (HBV DNA+, HBsAg-) are frequent in HIV+ patients, and the reactivation of an HBV infection can be dramatic. We describe a HIV+ patient (PhF) who was positive for α-HBs antibodies but experienced a strong and sudden reactivation of an occult HBV infection after glucocorticoid treatment. We focused on the molecular analysis of the HBV genome and HBsAg mutations that could explain this reactivation despite the presence of α-HBs. Full length HBV genomes were amplified from the patient’s serum using Rolling Circle Amplification (AAC, 52:3068 2008) followed by genomic PCR. Direct sequencing of the S gene showed that the HBV was genotype A2 (Ae) and 2 substitutions attracted our attention: sK122R and sD144E. Genomes were cloned and 2 were fully sequenced and functionally tested by transfection into HuH7 cells. One had the sK122R/sD144E mutations and the other had in addition sS154L. Both genomes are replication competent, but there were peculiar abnormalities in HBsAg expression that will be discussed. To see what patient’s serum was capable of recognizing, we used site-directed mutagenesis to produce genomes containing the mutations either singly or in combination. After transfection, cells were labeled with 35S-Met/Cys and labeled HBsAg secreted into the media were immunoprecipitated with the patient’s serum or control +ve and -ve sera. Both the patient’s serum and the +ve control serum readily recognize what we consider to be the WT HBsAg (sK122/D144/S154). However, introduction of sK122R or sD144E markedly reduces immunoprecipitation with the patient’s serum but not with the control serum. When both mutations are combined, recognition by the patient’s serum is completely abolished. We propose the following scenario: 1) acute infection with HBV and an α-HBs response directed against aa 122 and aa 144; 2) before viral clearance, a mutant (K122R?) emerged permitting continued low-level viral replication, the infection being controlled by α-HBs effective against the 2nd epitope; 3) glucocorticoid treatment stimulated HBV replication, leading to a conflict between immune response/viral replication resulting in the emergence of D144E. This study underlines the importance of virus/host/drug interactions.
The Nonsense Mediated mRNA Decay (NMD) protects the cell from the deleterious effects of truncated proteins by degrading the mRNA that exhibits a premature STOP codon (PTC). More than just a quality control pathway, the NMD is also currently described as a general posttranscriptional gene regulation pathway since it regulates non-mutated genes such as Gadd45α. On one side, INT6 has been identified by our team as a major actor of this mechanism, due to its interaction with the proteins UPF2 and CBP80 and its effect on the stability of PTC containing mRNA or other specific NMD regulated mRNA. On the other side, we demonstrated that INT6 is able to interact with Tax, the transcriptional activator of HTLV-1. This retrovirus is the etiological agent of the adult T cell Leukemia. Many observations lead to the conclusion that Tax plays a major role in the cellular transformation associated to HTLV. Moreover, due to the complex organisation of the HTLV-1 genome, the viral mRNA exhibits several key features of the usual NMD targets and so could be subjected to this decay pathway. As a consequence, a blockage of the NMD pathway by Tax targeting INT6 could increase the level of viral mRNA but also alter the genetic expression profil of an infected cell.

In order to verify the interference of Tax on the NMD, we first decided to decipher the network of interactions between this viral protein, INT6 and the UPFs, which are NMD core proteins, with immunoprecipitation experiments. We demonstrated that Tax prevents the correct interaction between INT6 and the UPF1/2 complex. These results suggest that Tax titrates INT6 and delocalises it out of reach from the UPF factors. We also observe that Tax interacts with UPF1 and UPF2 when these proteins are complexed together. Moreover Tax has been isolated with phospho forms of UPF1 that are usually needed during the NMD. The presence of Tax amongst NMD factors (including UPF3 and CBP 80) has also been observed in cells expressing the HTLV full provirus. If the identification of this network of interactions strongly suggests that Tax can modulate the efficiency of the NMD, we analysed the half life of mRNA in Jurkat cells expressing or not Tax: the half life of Gadd45α mRNA, known to be a target of the INT6 dependent NMD, is strongly stabilized in the presence of Tax. In order to confirm the inhibitory effect of Tax on the NMD we are currently experimenting the effect this protein onto the degradation of the β-globin mRNA holding a PTC (NS39) or not. Finally we are using WT/mutant forms of the full HTLV-1 provirus (in Tax, Rex or both together) in order to analyse the stability of the viral mRNA. The effect of a chemical NMD inhibitor will also be to be evaluated.

This work will help understanding if the interaction between Tax and the NMD core factors favours the HTLV-1 replication and the cell transformation.
E1E2-specific D32.10 antibodies inhibit the infection of the human HepaRG liver progenitor cells with serum hepatitis C virus particles (HCVsp) \textit{in vitro} and do predict complete recovery or sustained viral response to antivirals in the infected patients \textit{in vivo}.

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To study the neutralizing capability of the E1E2-specific D32.10 monoclonal antibody (mAb), an \textit{in vitro} direct cell-binding assay and an infection system of the human HepaRG cell line were developed by using HCVsp. The HepaRG cells possess potent ability to acquire a mature hepatocyte phenotype. The E1E2-specific mAb D32.10 was shown to inhibit efficiently and specifically high affinity-interactions through glycosaminoglycans and the CD81 tetraspanin between HCVsp and HepaRG cells with an IC50 = 0.5 µg/ml. This inhibition was more efficient when E1E2-positive enveloped HCVsp were used selectively for binding studies (IC50 < 0.5 µg/ml). Establishment of infection, replication and propagation of HCVsp were shown to depend on the proliferation/differentiation stage of HepaRG cells. Persistent HCV infection in HepaRG cells could be obtained with production of E1E2/RNA(+) infectious HCV particles. Preliminary data showed a complete early inhibitory effect of the D32.10 mAb on virion RNA production in HepaRG culture supernatants (95% at D14 and 80% at D21 post-infection). Furthermore, the detection of the anti-E1E2/D32.10-like antibodies during natural HCV infection demonstrated significant prevalence (90%) of these antibodies in patients who recovered spontaneously from HCV infection with high titers compared to patients with chronic hepatitis C, and in patients who are sustained virological responders compared to non responders to antivirals. Kinetic analyses revealed that the anti-E1E2/D32.10-like humoral response appeared very early with high titers (≥ 1/1000) and was associated with complete virus eradication. The positive and negative predictive values for complete spontaneous recovery (87-88%) or response to antiviral therapy (87-86%) are diagnostic accuracy. The anti-E1E2/D32.10-like antibodies may thus predict the outcome of HCV infection and constitute a new relevant HCV serological marker for the diagnosis. Convergence of \textit{in vitro} and \textit{in vivo} data strongly support the neutralizing activity of the D32.10 mAb, and therefore the potential immunotherapeutic applications of this unique anti-E1E2 mAb.
Study of CD150 expression in tumors of the central nervous system as a target for measles virus oncolytic treatment

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CD150 (IPO-3/SLAM) is a member of CD2 family of the immunoglobulin superfamily of surface receptors. This receptor is expressed on thymocytes, activated T and B lymphocytes, dendritic cells and monocytes. CD150 was also found on malignant cells at leukemias and lymphomas, mainly with activated cell phenotype. However, it is still little known about CD150 expression in non-lymphoid cells, especially in tumors. CD150 is one of two identified receptors for measles virus (MV). CD46 is a receptor for vaccine MV strains while CD150 is a receptor for both wild-type and vaccine MV strains. MV is considered as a promising novel antitumor agent in the treatment of gliomas. These tumors of the central nervous system are often resistant to the classical treatment or localized in the regions where surgery is difficult to perform, requiring therefore, development of new therapeutic approaches. Specific targeting of CD150 may allow a new, potentially more efficient oncolytic treatment of gliomas.

The aim of our work was to study the expression of CD150 in primary CNS tumors and in human glioma cell lines and to test susceptibility of glioma cells to MV infection. Immunohistochemical study of primary human CNS tumors with anti-CD150 antibody revealed CD150 expression in diffuse astrocytoma, anaplastic astrocytoma, glioblastoma, anaplastic oligoastrocytoma (50-80% of cases). Expression of CD150 in medulloblastoma, ependymoma and human normal brain tissues was not found. We then analyzed human glioma cell lines using RT-PCR and immunostaining and found differential expression of CD150 on mRNA and protein level. Only A172 cell line highly expressed the full transmembrane form of CD150 on mRNA level but the protein expression was not detected. U87, U343, NCH89 and NCH92 cells showed the low level of CD150 expression on both protein and mRNA levels (extracellular and transmembrane domains only). We then tested several glioma cell lines for the susceptibility to the infection with MV of vaccine and wild type strains. We observed only small level of wild type MV production in U343 cells but did not found any cytopathic effect in any of glioma cell lines.
However, vaccine strain of MV showed high level of viral particle production and strong cytopathic effect. Infection of U343 cells with vaccine MV resulted in MV hemagglutinin (H-MV) expression on the surface of the majority of cells, while after wild type MV infection H-MV was expressed on the surface of only small population of cells. Our results show that wild type MV can enter human glioma cells but does not cause the syncytia formation and cell lysis. It could be explained with the low level of the CD150 receptor expression on the surface of these cells. We are currently developing primary glioma short-term cell cultures, which may be closer to the in vivo conditions. Further studies may open new perspectives towards the development of potential oncolytic measles virus applications as novel antitumor agents, especially in the treatment of gliomas.
Preclinical studies on myrcludex B, a novel entry inhibitor for hepatitis B- and hepatitis delta virus (HDV) infections

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Background: The currently approved therapies for chronic Hepatitis B either block reverse transcription (RT) of the HBV-pregenomic RNA in infected hepatocytes or stimulate innate or adaptive immune responses by IFNa/pegIFNa. Although these drugs reduce HBV serum titres efficiently and lower the risk for long term liver failure, they are generally non-curative. Moreover, prolonged virus replication at reduced levels in the presence of RT-inhibitors results in selection of resistant mutants which can enter hepatocytes and lead to de novo cccDNA synthesis. We have previously shown that HBV L-protein-derived lipopeptides efficiently block HBV entry into hepatocytes in vitro and in vivo.

Methods: Using the HBV/HDV-susceptible HepaRG cell line as an in vitro infection system we defined an optimized inhibitory HBV-preS-lipopeptide designated “Myrcludex B”. This lead substance was synthesized according to GMP standards and used in preclinical trials (efficacy studies against different HBV genotypes, in vitro binding studies in primary hepatocytes of different species, pharmacokinetic and toxicity studies in rats and dogs).

Results: Myrcludex B is derived from 47 amino acids of the HBV L-protein and is N-terminally myristoylated. Its IC50 in vitro is in the picomolar range. Pharmacokinetic studies revealed that the peptide binds to serum albumin as a transporter and targets differentiated hepatocytes of even non HBV-susceptible animals with extraordinary selectivity. This suggests the presence of a species-comprehensive but hepatocyte-specific receptor. Mutational analyses showed that both, myristoylation and a conserved sequence motif are crucial for receptor interaction. Remarkably, HBV-receptor-inactivation by Myrcludex B is achieved at concentrations below the saturation of specific binding sites indicating a cooperative mechanism of action. Relevant animal models were identified and used for toxicology studies. Systemic administration up to 6 months duration revealed that Myrcludex B has a very low potential for toxicity and a broad therapeutic range.

Conclusion: Myrcludex B is a novel antiviral drug that targets and inactivates the HBV-preS1-specific receptor. Its potency to block HBV and HDV infection at picomolar concentrations combined with its excellent pharmacokinetic properties and its low toxicity makes it a promising additional therapeutic option for acute and chronic HBV and HDV infections.
Background: Ovine Pulmonary Adenocarcinoma (OPA) is an infectious lung cancer induced by the Jaagsiekte Sheep Retrovirus (JSRV). OPA shares strong clinical, radiological and histopathological features with a specific form of human cancer, the bronchioalveolar cancer (BAC) for which a viral etiology has not yet been identified. JSRV is unique among retroviruses to induced transformation of lung epithelial cells. It targets lung epithelial cells i.e. type II pneumocytes in the alveoli and Clara cells in the bronchioli. JSRV does not encode for an oncogene and tumoral transformation is not induced by proviral integration in the host genome. It is well established that the JSRV viral envelope protein is oncogenic by itself, due to YXXM motif sequence on the transmembrane glycoprotein (TM) that activates the PI3K/Akt and MAPK pathways. However, the molecular mechanisms triggered by this PI3K/Akt and MAPK activation pathways are not still elucidated. In order to better understand the precise molecular mechanisms triggered by the PI3K/Akt and MAPK activation, transcriptional profiles of OPA tissues were performed, in comparison to normal ovine lung tissue.

Results: Total RNA were extracted from OPA tissues and in vitro cell cultures established from OPA tissues, processed and hybridized of the Affymetrix GeneChip bovine Genome arrays. Following normalization of microarray data, analysis revealed 461 differentially expressed genes (>2 FC). Clustering analysis coupled to functional analysis using Ingenuity Pathway Analysis (IPA) system, identified several biological processes affected by OPA, including inflammation, cell organization and biogenesis, cell growth and maintenance, senescence inhibition and cell proliferation processes. Among these genes, the up-regulation of five oncogenes (CXCL2, S100G, AGR2, FGF and IGFBP3) and the down-regulation of eight tumor suppressor genes (GNG11, SNAI2, MYLK, NDN, FABP3, IGFBP4, RARRES, and EFNA5) were confirmed by real time PCR (P<0.05).

Conclusion: From these results, we concluded that these tumor suppressor genes are critically involved in OPA, and we focused our work on the methylation status of these genes.
Interaction between E7 protein of cutaneous Human Papillomavirus type 38 and eukaryotic elongation factor 1A regulates actin cytoskeleton structure

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IARC - France

The roles of the beta cutaneous human papillomavirus (HPV) in the development of non-melanoma skin cancers have not been clearly demonstrated. Our laboratory has previously demonstrated that the cutaneous HPV38 E6E7 could immortalize human keratinocytes in vitro, and that transgenic mice expressing E6 & E7 of HPV38 exhibit a high susceptibility to chemical-induced skin cancer. Mechanistically, HPV38 E6E7 induces an accumulation of specific forms of p53 that mediate the expression of its own inhibitor delta Np73, in contrast to the mucosal HPV16 E6 which targets p53 for degradation.

To further delineate the role of viral oncoproteins in HPV38-mediated carcinogenesis, GST-pulldown assay combined with mass spectrometry was performed to identify cellular proteins that interact with the HPV38E7. Human eukaryotic elongation factor 1A (eEF1A) was identified and the interaction between its two isoforms and HPV38E7 was confirmed by GST-pulldown, co-immunoprecipitation and subcellular colocalization. Remarkably, exogenous expression of eEF1A isoforms induced robust actin remodelling with increasing actin stress fibers, while over-expression of HPV38E7, on the opposite, not only destroyed actin stress fibers, but also reverse the eEF1A-induced actin fiber formation. Furthermore, we have found that besides eEF1A, HPV38E7 also interacts with F-actin, and that HPV38E7 competes with eEF1A for actin fiber interaction in vivo. Functionally, HPV38E7 down-regulates the RhoGTPase activities and blocks the RhoGTPase activation induced by eEF1A. Moreover, MEK kinase inhibitors could rescue the actin fiber loss induced by HPV38E7, indicating the roles of MEK/ERK pathway in HPV38E7-mediated actin filament disruption. Taken together, our data indicate that HPV38E7 negatively regulates the effects of eEF1A on actin cytoskeleton. This may account for the HPV38E7-mediated cellular immortalization and transformation.
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