



## Lecture

The search for infectious causes of human cancers: Where and why<sup>☆</sup>

Harald zur Hausen

Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

## A B S T R A C T

Slightly more than 20% of the global cancer burden can presently be linked to infectious agents, including viruses, bacteria and parasites. This manuscript analyzes reasons for their relatively late discovery and highlights epidemiological observations that may point to an involvement of additional infectious agents in specific human cancers. Emphasis is placed on hematopoietic malignancies, breast and colorectal cancers, but also basal cell carcinomas of the skin and lung cancers in non-smokers.

© 2009 Elsevier Inc. All rights reserved.

## Introduction

*Present state of the global cancer burden*

Presently a larger number of infectious agents have been identified which either cause or contribute to specific human cancers (reviewed in zur Hausen, 2006). They include two members of the herpes virus family, Epstein–Barr virus and human herpesvirus type 8, high risk and low risk human papillomaviruses (HPV), Hepatitis B and C viruses, a recently identified human polyomavirus, Merkel cell polyomavirus (Feng et al., 2008), the human T-lymphotropic retrovirus type 1 (HTLV-1), and human immunodeficiency viruses (HIV) types 1 and 2. In addition, human endogenous retroviruses have been suspected to play a role in human cancers. Besides viruses, other pathogens have also been identified. They include the bacterium *Helicobacter pylori*, a major contributor to gastric cancer, and parasitic infections, here in particular *Schistosoma hematobium*, a major cause of bladder cancer in Egypt, and liver flukes. The latter, *Opisthorchis viverrini* and *Clonorchis sinensis*, are important factors for cholangiocarcinomas and hepatocellular carcinomas in South-eastern Thailand and Southern China. Fig. 1 shows an estimate of the present contribution of infectious agents to the global cancer incidence.

It is important to note that there exist vast gender differences in the global role of papillomaviruses in human cancers. This is mainly due to the role of this virus family in the induction of cancer of the cervix. More than 50% of cancers linked to infections in females are caused by HPV infections. In males only approximately 4.3% of cancers have been linked to this virus family.

**Problems in identifying infectious agents involved in human cancer induction***Why has it been so difficult to identify infectious agents as causative factors for human tumors?*

The search for an infectious cause of at least some human cancers dates back to the second half of the nineteenth century (reviewed in zur Hausen, 2006). Yet, the first hints for a role of infectious agents in human cancers date back to the beginning of the 20th century, when *Schistosoma* infections in Egypt and liver flukes in Eastern Europe and Asia were suspected to play a role in the development of bladder and liver cancers. In spite of intensive search, it took approximately 65 additional years before further evidence was obtained, namely by linking a specific virus, Epstein–Barr virus, to two human cancers, Burkitt's lymphoma and nasopharyngeal carcinoma. During the past three or four decades progress has been more rapid, linking presently about 20% of the global cancer incidence to infectious events.

Why has it been so difficult to identify infectious agents as causative factors for human cancers? Several reasons seem to provide an explanation:

1. Because no human cancer arises as the acute consequence of infection. The latency periods between primary infection and cancer development are frequently in the range of 15 to 40 years. The X-chromosome-linked lymphoproliferation (XLLP) represents a rare exception. Based on a specific host cell mutation, Epstein–Barr virus here causes an acute lymphoproliferative disease.
2. Besides some rare exceptions, no synthesis of the infectious agents occurs in cancer cells.
3. Most of the infections linked to human cancers are common in human populations, they are ubiquitous. They were present during the whole human evolution. Yet, only a small proportion of infected individuals develops the respective cancer type.
4. Mutations in host cell genes or within the viral genome are mandatory for malignant conversion.

<sup>☆</sup> This manuscript covers the contents of the Nobel Lecture presented at the Karolinska Institute, Stockholm, Sweden on December 7, 2008, and it is reprinted with the permission of The Nobel Foundation. © The Nobel Foundation 2008.

E-mail address: [zurhausen@dkfz-heidelberg.de](mailto:zurhausen@dkfz-heidelberg.de).

**Cancers due to 5 infections correspond to 18.6% of total cancer incidence**

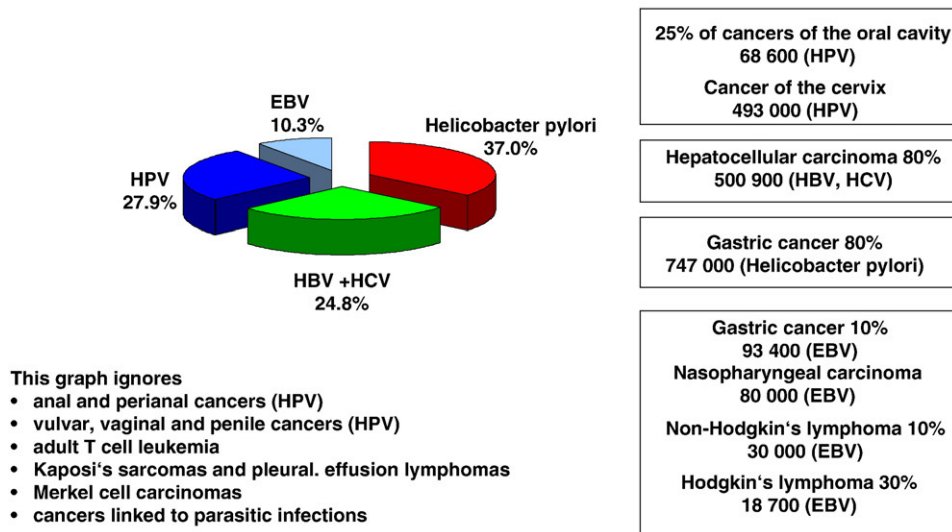


Fig. 1. Estimated annual global cancer incidence due to infections (with inclusion of data from Parkin et al., 2002, modified from zur Hausen, 2006).

5. Chemical (e.g. aflatoxin) and physical carcinogens (e.g. ultraviolet-light in Epidermodysplasia verruciformis) act usually as mutagens. They facilitate the selection of specific mutations and frequently act synergistically with carcinogenic infectious agents.
6. Some infectious agents act as indirect carcinogens, without persistence of their genes within the respective cancer cells (HIV, *H. pylori*, *S. hematobium*, Hepatitis C and B).

Among all these factors, the ubiquity of most of these infections and the long time periods required for malignant transformation were the main reasons for the remarkable difficulties in identifying their carcinogenic functions.

*Epidemiology provided hints for a successful search*

*Geographic coincidence*

Geographic coincidence of a specific infection (Hepatitis B) and of liver cancer led to the original suspicion that this infection may predispose to the subsequent development of hepatocellular carcinomas (reviewed in zur Hausen, 2006). The additional contribution of a chemical carcinogen was also suspected based on similar observa-

tions. Fig. 2 reveals the geographic distribution of Hepatitis B virus infections and hepatocellular carcinomas.

Geographic clustering of specific cancers may, however, also result from other causes: countries with a high rate of heavy smokers also experience a high incidence of lung cancer. The intensive solar exposure of Caucasian populations in Australia, South Africa and South America is responsible for a high percentage of skin cancer patients.

*Regional clustering of cases*

Regional clustering of specific cancer types triggered some investigations on a potential role of infectious agents in these malignant proliferations. Burkitt's lymphoma in equatorial Africa represents one of the most illustrative examples. Burkitt noted the apparent dependence of tumor incidence on climatic conditions, altitude and described the regional correlation with holoendemic *Plasmodium falciparum* infections (Burkitt, 1962). As a consequence he speculated that the tumor might be due to a viral infection, transmitted by an arthropod vector, possibly the same carrying malaria parasites.

Nasopharyngeal carcinoma, occurring at high frequency in specific regions of South East Asia represents another example. Adult T-cell leukemia in the coastal regions of Southern Japan, cholangiocar-

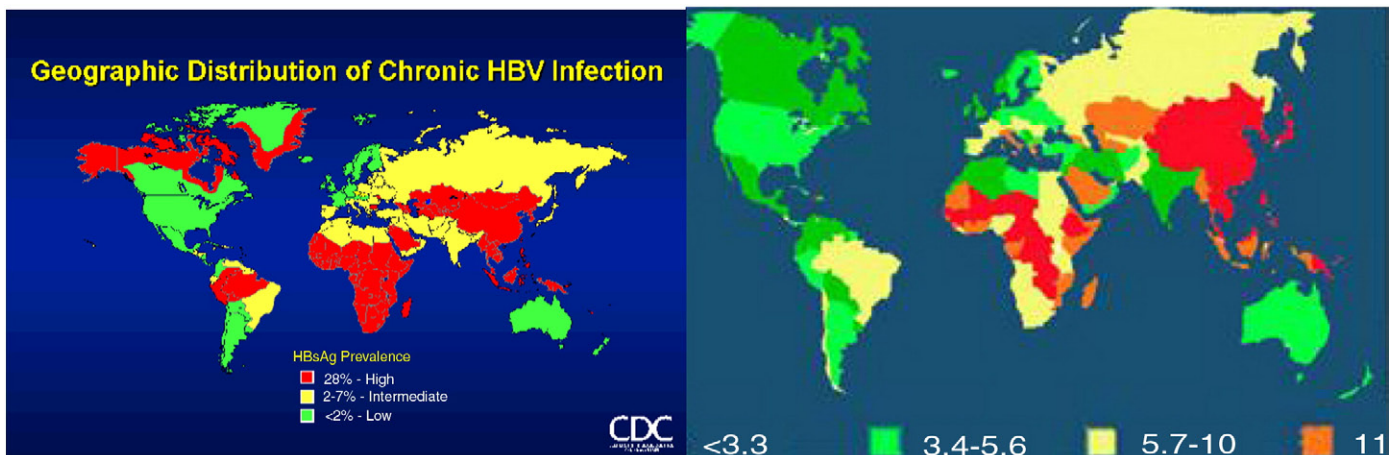


Fig. 2. Geographic distribution of Hepatitis B infections (left) and of hepatocellular carcinomas (right). Modified from figures provided by CDC and Globocan 2002.

cinomas in South East Thailand, and bladder cancer in the Nile Delta or along the Nile River also raised early suspicions for an infectious origin. These observations resulted in speculations; they could not prove the underlying hypothesis by themselves.

#### Dependence on sexual contacts

If one disregards the occurrence of scrotum cancer in chimney sweepers, the early studies of Rigoni-Stern in Verona, Italy, pointing in 1842 to a role of sexual contacts in the causation of cervical cancer, represent a particularly interesting example of suspected contact transmission of a human cancer. It took another 140 years before the viral infections were identified causing this frequent cancer in women. These observations led to the identification of additional anogenital and oral cancers linked to the same virus infections.

#### Cancers arising under immunosuppression

Epidemiological surveys identified immunosuppression as a condition resulting in the appearance of remarkably specific forms of cancer. Many of those malignancies have by now been shown to be caused by reactivated viruses, whose oncogenic potential is usually suppressed by immunological reactions. The most prominent tumors arising here are Epstein–Barr virus caused B-cell lymphomas, Kaposi's sarcomas linked to human Herpesvirus type 8 reactivation, and Merkel cell carcinomas of the skin associated with a novel polyomavirus. The initial discovery of the viral origin of cervical cancer and its precursor lesions was not based on the moderately enhanced incidence under immunosuppression. Specific types of common warts as a non-malignant proliferative condition also occur at high frequency in immunosuppressed patients, mainly containing of genus-Beta papillomaviruses. The viral origin of basal and squamous cell carcinomas of the skin, frequently found in these patients, remains up to now controversial.

#### Mechanistic aspects of cancer induction by infections

Fig. 3 lists identified mechanisms by which infections may contribute to cancer development. The expression of specific viral oncogenes as a mandatory precondition for the maintenance of the malignant phenotype has been identified as a direct contributions to human carcinogenesis (reviewed in zur Hausen, 2006). A novel mode of direct viral carcinogenesis has probably been identified in Merkel cell carcinomas, where functional inactivation of the helicase part of

the large T-antigen of the Merkel cell polyomavirus renders the viral DNA replication-incompetent (Shuda et al., 2008). Viral DNA persisting in normal tissues seems to retain replication-competence.

The most prominent indirect infectious carcinogens are agents causing immunosuppression or inducing via inflammatory reactions reactive oxygen species. Whereas the mechanism of immunosuppression induced by human immunodeficiency viruses (HIV) or after organ transplantation is reasonably well understood, the accurate mechanism by which Hepatitis B and C viruses, *H. pylori* and carcinogenic parasitic infections contribute to cancer still remains somewhat obscure.

#### Where is it worthwhile to search for an infectious etiology of human cancers not yet linked to infections?

When we summarize infectious agents that have been discovered during the past 15 years, it is interesting to note that several novel viruses belonging into potentially carcinogenic virus families have been identified even during the past 2 years (Fig. 4). This raises the suspicion that additional links to novel or already identified infectious agents to cancers will become apparent, hitherto not linked to infections. Thus, it appears worthwhile to search for cancer-related epidemiologic observations that may point to the involvement of infectious agents in cancers hitherto not linked to infections. The following will summarize some hypotheses and considerations based on these reports.

#### Cancers occurring under immunosuppression

A review published by Vajdic et al. (2006) demonstrates a larger number of cancers occurring at increased frequency under immunosuppression after kidney transplantation. Kaposi's sarcoma mainly found in HIV-infected patients stands out and is found about 200-fold increased in these patients in comparison to non-infected controls (Fig. 5). The most interesting part of Fig. 5 appears to be the 7–8-fold higher rate of vulva and penile cancer in comparison to cancer of the cervix. The vast majority of cervical cancers are caused by high risk human papillomavirus (HPV) infections. In vulva and penile cancers only 30–50% seems to be linked to the same HPV infections. The etiology of 50–70% of these cancers is unknown. Interestingly, the age distribution of HPV-positive and HPV-negative vulva and penile cancers differs in that the negative tumors regularly occur in older

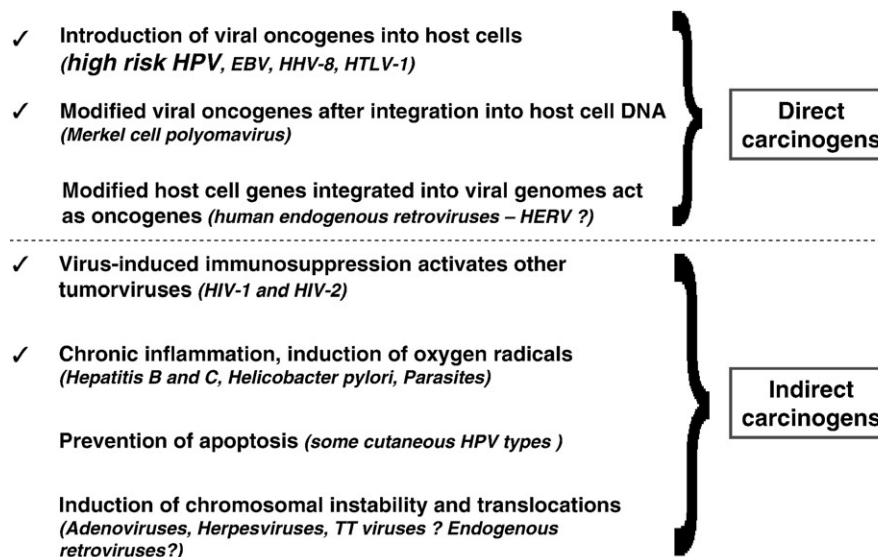


Fig. 3. Summary of identified mechanisms by which infections either directly or indirectly contribute to carcinogenesis. Mechanistic contributions of infections to human cancers have been marked.

Year	Virus	Symptoms	Natural Host
1994	Sabia virus	Hemorrhagic fever	Rodents
1994	Hum. Herpesvirus 8	Kaposi's sarcoma	Humans
1994	Hendravirus	Encephalitis	Bats, horses
1997	Influenza H5N1	Avian flue	Birds
1997	TT viruses	?	Humans
1998	Nipah virus	Encephalitis	Bats, pigs
2003	SARS Coronavirus	SARS	Chinese bushcat
2005	Bocavirus (parvovirus)	Acute wheezing	Humans
2005	New coronavirus	Respiratory symptoms	Humans
2007	KI-polyomavirus	?	Humans
2007	WU-polyomavirus	?	Humans
2008	MC-polyomavirus	Merkel-tumor	Humans
2008	Lymphotrop. polyomavirus	Periph. blood PML patients	Humans

Within the same time period at least 30 novel types of human papillomaviruses have been identified

Fig. 4. "New" human pathogenic viruses 1994–2008. The light arrows identify important human pathogens or a whole novel virus family (TT viruses). The dark arrows point to established or potentially oncogenic virus isolates.

age groups. Thus, the negative group should require attention as possible candidates for an unknown viral etiology. Unidentified types of HPVs or novel polyomaviruses may represent interesting candidates. Salivary gland, eye, thyroid and tongue cancers should also deserve attention.

*Cancers not elevated or even reduced after immunosuppression*

*Breast cancer as an example*

Some cancers do not show an increased incidence during immunosuppression. Indeed, immunosuppression may even possess a protective effect for some of these tumors. Those cancers are shown in Fig. 6. Besides prostate, rectum and brain tumors, human breast cancer represents a particularly intriguing malignancy, because murine mammary cancer is also not increased under immunosuppression. This latter tumor is caused by a retrovirus infection, murine mammary tumor virus (MMTV).

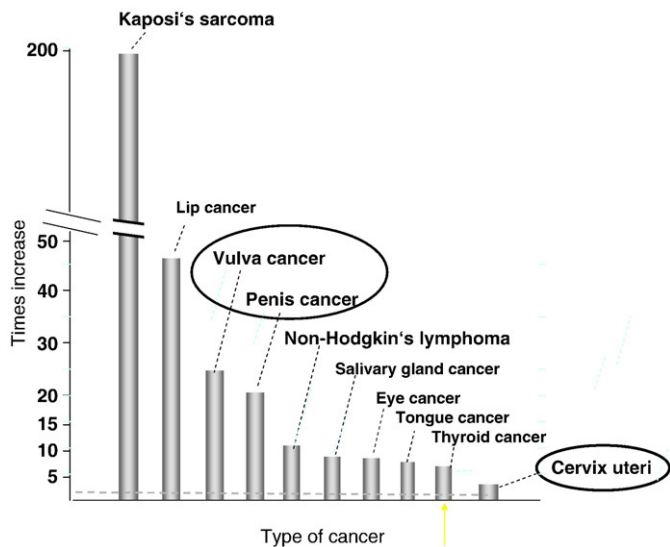


Fig. 5. Some of the most frequently occurring cancers occurring after kidney transplantation (modified from Vajdic et al., 2006). The dotted line indicates the incidence in immunocompetent patients.

In murine mammary tumors the mechanism of a slightly protective effect exerted by immunosuppression is partially understood (see review of zur Hausen, 2006). It is schematically outlined in Fig. 7. The primary infection occurs via the milk of the infected mother. The virus reaches the Peyer's patches where it infects B- and T-lymphocytes. Superantigen induction in the infected cells leads to reactive T-cell depletion and immunotolerance. The superantigen expressing cells produce high quantities of infectious MMTV; this substantially increases the risk for the infection of mammary tissue. Specific integration of the MMTV proviral DNA in the mammary cells emerges as the prime risk factor for the resulting mammary carcinomas. Immunosuppression of such infected animals apparently interferes with the emergence of superantigen-producing T- and B-lymphocytes and, as a consequence, suppresses virus production which in turn decreases the risk for cancer development.

Is it possible that a similar mechanism contributes to human mammary cancer? A few data seem to support this notion. They may point to a possible involvement of a specific subgroup of human endogenous retroviruses (HERV) in this malignancy. At least 8% of our genome consists of retroviral sequences acquired in the course of human evolution. Although the vast majority of these sequences do no longer reveal functional open reading frames, members of one subgroup HERV-K, which entered our germline approximately 800,000 years ago, are still able to code for complete, though non-

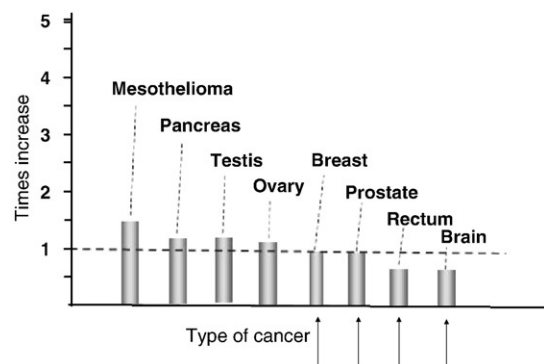


Fig. 6. Cancer incidence marginally or not affected during immunosuppression after kidney transplantation (modified from Vajdic et al., 2006).

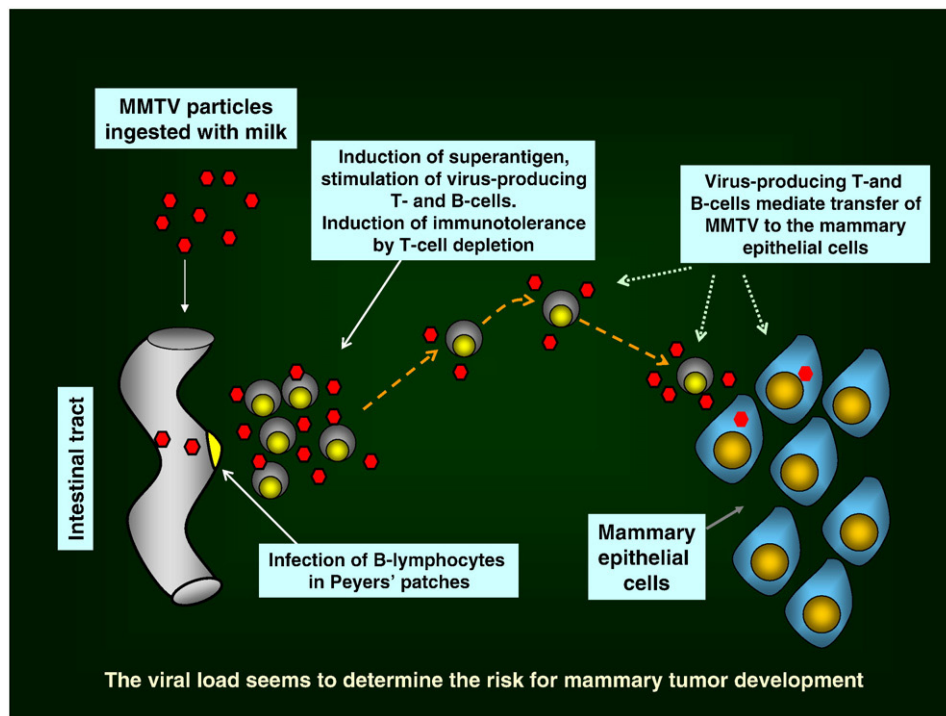


Fig. 7. Schematic outline of events following infection of newborn mice with murine mammary tumor virus (modified from zur Hausen, 2006).

infectious virus particles. Retroviral gag and env transcripts of the 22q11.21 region are found in these particles (Ruprecht et al., 2008). Correction of stop codons in HERV-K sequences resulted even in the re-constitution of infectious HERV-K viruses (Dewannieux et al., 2006, Lee and Bieniasz, 2007). HERV-K expression also becomes activated by other virus infections: HIV infections activate HERV-K sequences (Laderoute et al., 2007). Similarly Epstein–Barr virus infections result in the induction of HERV-K superantigen (Sutkowski et al., 2004, Meylan et al., 2005, Hsiao et al., 2006). Epstein–Barr virus containing Burkitt's lymphoma cells occasionally reveal particles strongly resembling retroviral type A structures upon induction by the tumor-promoting phorbol ester TPA (zur Hausen, unpublished). Typical structures are shown in Fig. 8.

Some recent reports may further stress a potential role of reactivated HERV-K viruses in the pathogenesis of human breast cancer: an antigen-specific immune response was demonstrated in breast cancer patients (Wang-Johanning et al. 2008). In addition, breast cancer patients, HIV-associated lymphomas, non-HIV-associated lymphomas, HIV-associated Hodgkin's lymphomas reveal about 7-fold elevated concentrations of HERV-K (HML-2) RNA in their plasma when compared to healthy controls (Contreras-Galindo et al. 2008). The RNA titers in lymphoma patients in remission returned to control values.

Although the available data seem to support a potential role of endogenous retroviruses in human breast cancer, they certainly do not prove it. Other agents may also contribute to at least a proportion of these cancers. A possible link of red meat consumption in relation to breast cancer and a potential involvement of other viral factors will be discussed in connection with a subsequent paragraph. Nevertheless, human breast cancer remains an interesting candidate for a viral etiology.

#### Cancer incidence influenced by infections

The risk for some cancers seems to be influenced by other infections which neither directly contribute to carcinogenesis nor induce long-lasting immunosuppression.

#### Basal cell carcinomas in pox scars

Fig. 9 reveals an example of multiple basal cell carcinomas arising after 20 years in a smallpox vaccination scar. This does not represent a solitary observation, since a larger number of basal cell carcinomas, but also melanomas, squamous cell carcinomas, and a few more rarely occurring malignancies (dermatofibrosarcoma protuberans, fibrosarcoma and malignant fibrous histiocytomas) have also been reported to occur in smallpox vaccination scars (Waibel and Walsh, 2006). They are summarized in Fig. 10.

Prior to the eradication of smallpox infections, vaccines against these infections were prepared by inoculating vaccinia virus into the scarified skin of calves and harvesting the skin crusts containing the vaccinia virus particles. It is possible that these preparations contained contaminating bovine viruses. Previously it has been demonstrated that vaccinia virus infections cause amplification of

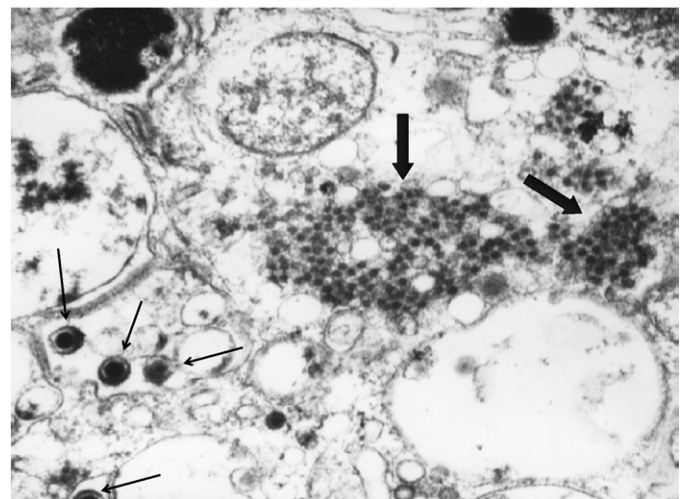


Fig. 8. Epstein–Barr virus particles (small arrows) and two clusters of A-type particle-like structures (big arrows) in a TPA-treated Burkitt's lymphoma cell.

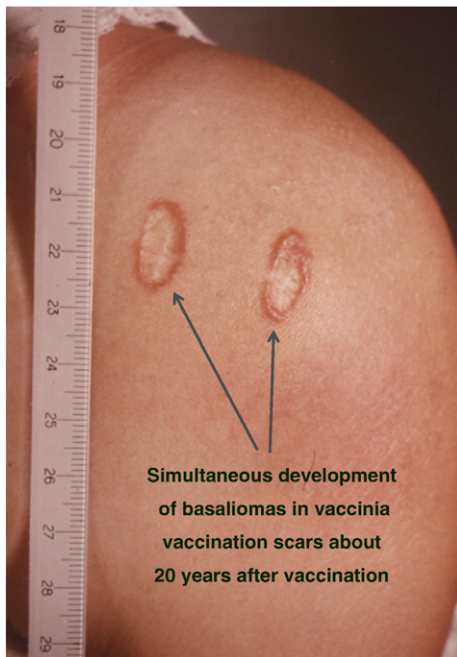


Fig. 9. Multifocal basal cell carcinomas in pox scars.

persisting polyoma-type virus genomes (Schlehofer et al., 1986). This may increase the likelihood for contaminations with bovine members of this virus family. Persisting papillomavirus DNA would be also affected in cells replicating vaccinia virus (Schmitt et al., 1989).

The published data permit several interpretations:

- Vaccinia virus infection of calf skin resulted in the activation of specific cattle viruses whose subsequent inoculation into humans as contamination represented a risk factor for subsequent local cancer development;
- Vaccinia virus infection of the human skin resulted in local activation of human potentially oncogenic viruses, increasing the risk for cancer development 20–60 years later;
- Early inflammatory reactions induced by this vaccination resulted in mutational events resulting in some cases in the simultaneous appearance of multifocal cancers.

Although still other interpretations remain possible, and basal cell carcinomas have also occasionally been observed in other non-vaccination scars, the observations described here should promote studies on a possible viral role in the initiation of these malignant proliferations.

#### Hematopoietic malignancies

As shown in Fig. 11, a number of human viruses turn out to be oncogenic when inoculated into newborn rodents. Intracerebral infections by JC virus are able to induce astrocytomas in adult owl monkeys (London et al., 1978). For obvious reasons the reverse question, whether specific animal viruses are also able to induce tumors in humans has not yet been carefully investigated (zur Hausen, 2001). Yet, we are living in close contact with domestic animals and regularly handle their products. This is particularly interesting because contact with cattle and consumption of red meat have been identified as risk factors for specific human malignancies. Contact with cattle has also frequently been considered as risk factor for hematopoietic malignancies, in particular childhood acute lymphocytic leukemias (reviewed in zur Hausen, 2009).

**Risk and protective factors.** In the following the reasons for considering childhood leukemias as potential candidates for an infectious etiology will be briefly summarized. A more detailed account has been published recently (zur Hausen, 2009). Some protective factors as well as several risk factors for this malignancy are presented in Fig. 12.

Rare infections during the first year of life are frequently reported as a risk factor for childhood leukemias (reviewed in zur Hausen, 2009). Conversely, multiple infections during this period emerge as a protective factor. These observations are underlined by correlative data: a high socioeconomic state represents a risk factor, whereas crowded household conditions and many siblings emerge as protective factors. Cattle's farming has been reported as an additional risk factor, whereas more than 6 months of breast feeding seem to reduce the risk.

Two additional sets of data deserve discussion: the frequent occurrence of specific chromosomal translocations in leukemic cells, often observed already prenatally (Greaves, 2003). The same types of chromosomal alterations have also been found in healthy individuals, though here their frequency appears to be very low. Another striking observation originates from the description of occasional small clusters of leukemic cases, specifically in regions where an influx of urban populations occurred in previously rural areas (reviewed in Kinlen, 2004).

**Possible explanations.** Three main hypotheses have been published to explain the epidemiological findings: Greaves (summarized in 2006) speculated that there exists an insufficient maturation state of the immune system in case of low exposure to infections. Preceding chromosomal translocations as the first event, followed by delayed infection "with an unspecified agent" should increase the risk for subsequent leukemic conversion. Alternatively, Kinlen (1995) proposed that sudden mixing of a population of low exposure to a putative leukemogenic agent (particularly in rural areas) with another

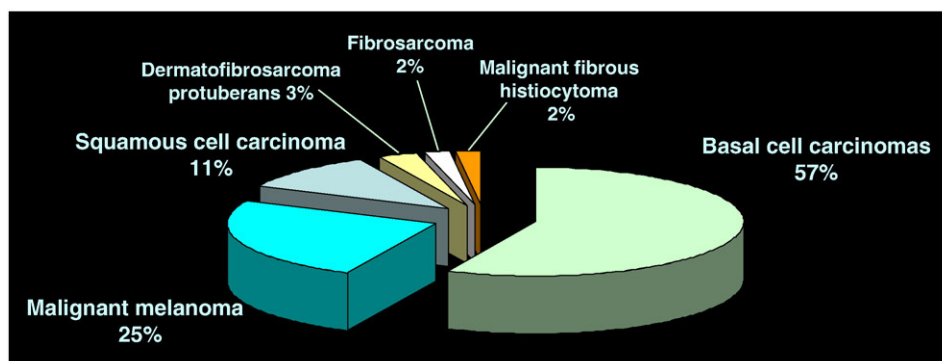


Fig. 10. Malignant tumors arising in vaccinia virus vaccination scars (modified from Waibel and Walsh, 2006).



Fig. 11. Some human viruses are carcinogenic for several animal species. Do animal viruses exist potentially carcinogenic in humans?

population originating from urban areas previously highly exposed to the incriminated agent, could promote an epidemic of the relevant infection. These hypotheses were supplemented by a further speculation, assuming that the protective effect of multiple infections during the first year of childhood was due to the reduction of the load with a putative leukemogenic agent by interferon production as outlined in Fig. 13 (zur Hausen and de Villiers, 2005, zur Hausen, 2009).

Reports on supertransforming properties of specifically replication-incompetent SV40 and murine polyomaviruses (Small et al., 1982, Roberge and Bastin, 1988), in addition to the recent demonstration of replication-incompetent Merkel cell polyomavirus in Merkel cell carcinomas (Shuda et al., 2008) resulted in an attempt to combine the three hypotheses, assuming that replication-incompetent polyoma-type viruses and high multiplicities at the time of initial infection represent an important precondition for an increased leukemogenic risk. The generation of replication-incompetent viral progeny seems

to depend on high multiplicities of infection and the co-infection of cells with both, replication-competent and incompetent genomes. The sole subsequent infection of a susceptible cell with a replication-incompetent genome may lead to the outgrowth of a leukemic clone. Susceptibility of a cell for this malignant conversion would require the previous or subsequent acquisition of a specific chromosomal translocation. These translocations also occur in healthy individuals, though at low frequency (Liu et al., 1994, Bell et al., 1995, Fuscoe et al., 1996, Roulland et al., 2006). They represent risk factors but are clearly not sufficient for cell transformation. They should activate the oncogene of the replication-incompetent virus. A synopsis of this hypothesis is presented in Fig. 14.

A polyoma-type virus infection would fit best for this model, although members of structurally related virus families might be also considered. Since a number of reports document elevated risks in families of cattle farmers and for an individual in close contact with

Repeatedly reported <u>protective factors</u> for childhood leukemias:	<u>Risk factors</u> for childhood leukemias
<div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">Multiple infections in early childhood</div> Underprivileged social status Crowded household, many siblings Inverse risk with birth order More than 6 months of breastfeeding	<div style="border: 2px solid red; padding: 5px; margin-bottom: 5px;">Rare infections during the first year of life</div> High socioeconomic status Prenatal chromosomal translocations Agricultural occupation of parents

Fig. 12. Protective and risk factors for childhood acute lymphoblastic leukemia.

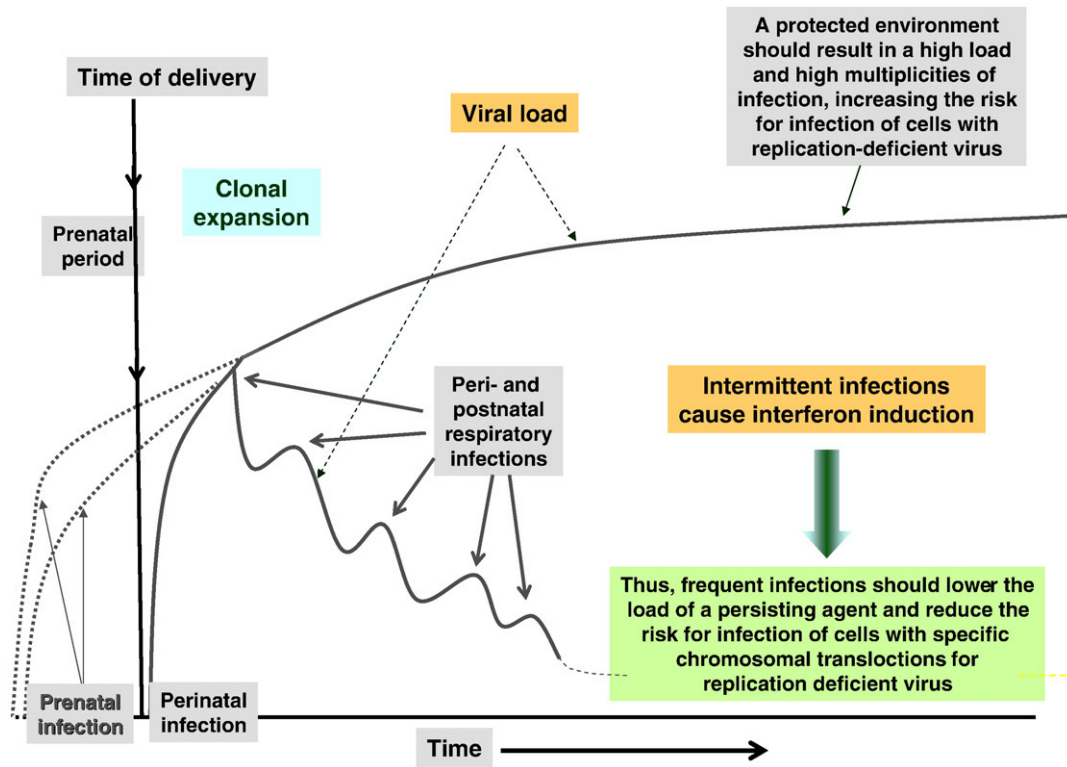


Fig. 13. Schematic outline of the target cell conditioning hypothesis. Interferon synthesis resulting from multiple infections in early childhood reduces the load of a persisting potentially leukemogenic agent and thus reduces the risk for malignant proliferation (adapted from zur Hausen and de Villiers, 2005).

cattle (reviewed in zur Hausen, 2009), at least part of childhood leukemias could be due to a native cattle virus. This virus should be replication-incompetent for human cells, but its oncogene may become activated in cells with specific chromosomal modifications. Since a number of reports also suggest human occupational risks of persons with communicative contacts (e.g. teachers, hair dressers),

other types of similar infections may be spread by human–human contacts (reviewed in zur Hausen, 2009).

It remains an interesting question to which extent other hematopoietic malignancies, like acute and chronic myelogenous leukemias, chronic lymphatic leukemias, B- and T-cell non-Hodgkin lymphomas, Epstein–Barr virus-negative Hodgkin lymphomas, and

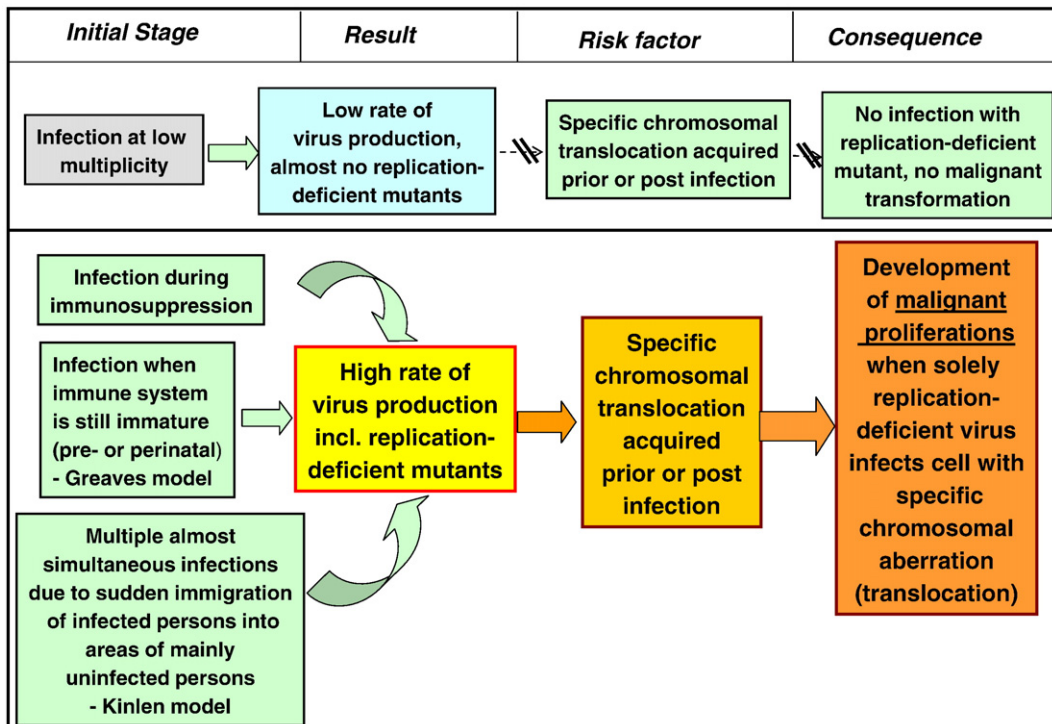


Fig. 14. Synopsis of the target cell conditioning model for childhood leukemia.





Fig. 15. Red meat cooked "raw".

multiple myelomas could be included in these considerations. Yet undefined polyomavirus-like particles have been electron microscopically demonstrated in trichodysplasia of a patient with non-Hodgkin's lymphoma (e.g. Osswald et al., 2007).

#### *Cancers potentially linked to animal–human transmission*

##### *Colorectal, breast and lung cancers*

A large number of reports consistently describe an increased risk for colorectal cancers related to a high consumption of red meat (reviewed in Kuhnle and Bingham, 2007, Santarelli et al., 2008). Recently this has also been noted for lung cancer in non-smokers (Cross et al., 2007, Hu et al., 2008, Lam et al., 2009) and, to a more limited degree less consistently, also for breast cancer (Taylor et al., 2007, Egeberg et al., 2008, Hu et al., 2008, Linos et al., 2008, Mignone et al., 2009). A correlation seems to exist in countries with a high rate of red meat consumption and a high risk for colorectal and breast cancer. Common and frequently cited interpretations of these observations are dietary factors. Carcinogenic N-nitroso compounds, heterocyclic amines and heterocyclic aromatic hydrocarbons arise during cooking, broiling or meat curing. Some of these compounds require metabolic activation prior to conversion into a carcinogenic form, as initially described by Sugimura and colleagues (Ohgaki et al., 1986). In addition, potentially carcinogenic nitrosyl haem and nitroso thiols have been reported to be significantly increased in feces following a diet rich in red meat (Cross et al., 2002).

In contrast to red meat, consumption of white meat, and here specifically chicken and other poultry meat has not been found to be associated with an elevated risk for colorectal or other cancers. It has been reported, however, that fried, grilled or smoked chicken meat contains equally high concentrations of heterocyclic aromatic hydrocarbons and other carcinogens arising in the preparatory steps prior to consumption (Yano et al., 1988, Kazerouni et al., 2001, Reinik et al., 2007). If this holds up and if no other hitherto unknown carcinogens are found specifically in red meat, these observations may require a fresh look at previous interpretations. In meat prepared medium or raw (Fig. 15), temperatures in the central portions do not exceed 55 to 65 °C. At least members of the polyoma- and papillomavirus families readily survive these temperatures without significant loss of their infectivity (Lelie et al., 1987, Sauerbrei and Wutzler, 2009). The only known bovine polyomavirus was initially identified as a contamination of fetal bovine sera, thus, it must have been present in the peripheral blood of yet unborn or newborn calves. Existing members of the polyomavirus family have been poorly studied in our domestic animals. These viruses are commonly non-oncogenic in their natural

hosts, but reveal carcinogenicity only in heterologous tissues. Presently 6 different genotypes of polyomaviruses have been identified in humans, but only one in cattle.

It is tempting to speculate that a hitherto unidentified bovine infectious agent with pronounced thermostability, replication-incompetent for human cells and possibly structurally related to the polyomavirus family, may play a role in colorectal cancer, potentially also in lung cancers of non-smokers and in breast cancer. This could be interpreted to mean that the described chemical carcinogens arising during cooking or curing processes are not sufficient for the induction of the respective cancers. In the case of red meat consumption they may, however, interact with viral agents, present in red, but not in white meat.

#### **Conclusions**

Although we know that presently slightly more than 20% of the global cancer incidence is linked to infectious events, some epidemiological observations suggest that this percentage will increase in the future. The recognition that no cancer linked to infections develops without additional modifications within the host cell genome permits the speculation that even cancers with well established chromosomal modifications deserve a careful analysis for an additional involvement of infectious agents. Prime malignancies suggested here as candidates for potential links with infections are hematopoietic malignancies, particularly childhood lymphoblastic leukemias, Epstein–Barr virus-negative Hodgkin's lymphomas, basal cell carcinomas of the skin, and breast, colorectal and a subgroup of lung cancers. Although still hypothetical, this proposal is accessible to experimental verification. Even if only one of these speculations turns out to be correct, this would have profound implications for the prevention, diagnosis and hopefully also for therapy of the respective malignancy.

#### **References**

- Bell, D.A., Liu, Y., Cortopassi, G.A., 1995. Occurrence of bcl-2 oncogene translocation with increased frequency in the peripheral blood of heavy smokers. *J. Natl. Cancer Inst.* 87, 223–224.
- Burkitt, D., 1962. A children's cancer dependent on climatic factors. *Nature* 194, 232–234.
- Contreras-Galindo, R., Kaplan, M.H., Leissner, P., Verjat, T., Ferlenghi, I., Bagnoli, F., Giusti, F., Dosik, M.H., Hayes, D.F., Gitlin, S.D., Markovitz, D.M., 2008. Human endogenous retrovirus K (HML-2) elements in the plasma of people with lymphoma and breast cancer. *J. Virol.* 82, 9329–9336.
- Cross, A.J., Pollock, J.R., Bingham, S.A., 2002. Red meat and colorectal cancer risk: the effect of dietary iron and haem on endogenous N-nitrosation. *IARC Sci. Publ.* 156, 205–206.
- Cross, A.J., Leitzmann, M.F., Gail, M.H., Hollenbeck, A.R., Schatzkin, A., Sinha, R., 2007. A prospective study of red and processed meat intake in relation to cancer risk. *PLoS Med.* 4, e325.
- Dewannieux, M., Harper, F., Richaud, A., Letzelter, C., Ribet, D., Pierron, G., Heidmann, T., 2006. Identification of an infectious progenitor for the multiple-copy HERV-K human endogenous retroelements. *Genome Res.* 16, 1548–1556.
- Egeberg, R., Olsen, A., Autrup, H., Christensen, J., Stripp, C., Tetens, I., Overvad, K., Tjønneland, A., 2008. Meat consumption, N-acetyl transferase 1 and 2 polymorphism and risk of breast cancer in Danish postmenopausal women. *Eur. J. Cancer Prev.* 17, 39–47.
- Feng, H., Shuda, M., Chang, Y., Moore, P.S., 2008. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 319, 1096–1100.
- Fusco, J.C., Setzer, R.W., Collared, D.D., Moore, M.M., 1996. Quantification of t(14;18) in the lymphocytes of healthy adult humans as a possible biomarker for environmental exposures to carcinogens. *Carcinogenesis* 17, 1013–1020.
- Greaves, M., 2003. Pre-natal origins of childhood leukemia. *Rev. Clin. Exp. Hematol.* 7, 233–245.
- Greaves, M., 2006. The causation of childhood leukemia: a paradox of progress? *Discov. Med.* 6, 24–28.
- Hsiao, F.C., Lin, M., Tai, A., Chen, G., Huber, B.T., 2006. Cutting edge: Epstein–Barr virus transactivates the HERV-K18 superantigen by docking to the human complement receptor 2 (CD21) on primary B cells. *J. Immunol.* 177, 2056–2060.
- Hu, J., La Vecchia, C., DesMeules, M., Negri, E., Mery, L., 2008. Canadian Cancer Registries Epidemiology Research Group. Meat and fish consumption and cancer in Canada. *Nutr. Cancer* 60, 313–324.
- Kazerouni, N., Sinha, R., Hsu, C.H., Greenberg, A., Rothman, N., 2001. Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. *Food Chem. Toxicol.* 39 (5), 423–436 (May).
- Kinlen, L.J., 1995. Epidemiological evidence for an infective basis in childhood leukaemia. *Br. J. Cancer.* 71, 1–5.

- Kinlen, L.J., 2004. Childhood leukemia and population mixing. *Pediatrics* 114 (1), 330–331 (Jul).
- Kuhnle, G.G., Bingham, S.A., 2007. Dietary meat, endogenous nitrosation and colorectal cancer. *Biochem. Soc. Trans.* 35, 1355–1357.
- Laderoute, M.P., Giulivi, A., Larocque, L., Bellfroy, D., Hou, Y., Wu, H.X., Fowke, K., Wu, J., Diaz-Mitoma, F., 2007. The replicative activity of human endogenous retrovirus K102 (HERV-K102) with HIV viremia. *AIDS* 21, 2417–2424.
- Lam, T.K., Cross, A.J., Consonni, D., Randi, G., Bagnardi, V., Bertazzi, P.A., Caporaso, N.E., Sinha, R., Subar, A.F., Landi, M.T., 2009. Intakes of red meat, processed meat, and meat mutagens increase lung cancer risk. *Cancer Res.* 69, 932–939.
- Lee, Y.N., Bieniasz, P.D., 2007. Reconstitution of an infectious human endogenous retrovirus. *PLoS Pathog.* 3 (1), e10.
- Lelie, P.N., Reesink, H.W., Lucas, C.J., 1987. Inactivation of 12 viruses by heating steps applied during manufacture of a hepatitis B vaccine. *J. Med. Virol.* 23, 297–301.
- Linos, E., Willett, W.C., Cho, E., Colditz, G., Frazier, L.A., 2008. Red meat consumption during adolescence among premenopausal women and risk of breast cancer. *Cancer Epidemiol. Biomark. Prev.* 17, 2146–2151.
- Liu, Y., Hernandez, A.M., Shibata, D., Cortopassi, D.A., 1994. BCL2 translocation frequency rises with age in humans. *Proc. Natl. Acad. Sci. U. S. A.* 91, 8910–8914.
- London, W.T., Houff, S.A., Madden, D.L., Fuccillo, D.A., Gravell, M., Wallen, W.C., Palmer, A.E., Sever, J.L., Padgett, B.L., Walker, D.L., Zuerlein, G.M., Ohashi, T., 1978. Brain tumors in owl monkeys inoculated with a human polyomavirus (JC virus). *Science* 201, 1246–1249.
- Meylan, F., De Smedt, M., Leclercq, G., Plum, J., Leupin, O., Marguerat, S., Conrad, B., 2005. Negative thymocyte selection to HERV-K18 superantigens in humans. *Blood* 105, 4377–4382.
- Mignone, L.L., Giovannucci, E., Newcomb, P.A., Titus-Ernstoff, L., Trentham-Dietz, A., Hampton, J.M., Orav, E.J., Willett, W.C., Egan, K.M., 2009. Meat consumption, heterocyclic amines, NAT2, and the risk of breast cancer. *Nutr. Cancer* 61, 36–46.
- Ohgaki, H., Hasegawa, H., Kato, T., Suenaga, M., Ubukata, M., Sato, S., Takayama, S., Sugimura, T., 1986. Carcinogenicity in mice and rats of heterocyclic amines in cooked foods. *Environ. Health Perspect.* 67, 129–134.
- Osswald, S.S., Kulick, K.B., Tomaszewski, M.M., Sperling, L.C., 2007. Viral-associated trichodysplasia in a patient with lymphoma: a case report and review. *J. Cutan. Pathol.* 34, 721–725.
- Parkin, D.M., Bray, F., Ferlay, J., Pisani, P., 2002. Global cancer statistics, 2002. *CA Cancer J. Clin.* 55, 74–108.
- Reinik, M., Tamme, T., Roasto, M., Juhkam, K., Tenno, T., Kiis, A., 2007. Polycyclic aromatic hydrocarbons (PAHs) in meat products and estimated PAH intake by children and the general population in Estonia. *Food Addit. Contam.* 24, 429–437.
- Roberge, C., Bastin, M., 1988. Site-directed mutagenesis of the polyomavirus genome: replication-defective large T mutants with increased immortalization potential. *Virology* 162, 144–150.
- Roulland, S., Lebailly, P., Lecluse, Y., Heutte, N., Nadel, B., Gauduchon, P., 2006. Long-term clonal persistence and evolution of t(14;18)-bearing B cells in healthy individuals. *Leukemia* 20, 158–162.
- Ruprecht, K., Ferreira, H., Flockerzi, A., Wahl, S., Sauter, M., Mayer, J., Mueller-Lantsch, N., 2008. Human endogenous retrovirus family HERV-K(HML-2) RNA transcripts are selectively packaged into retroviral particles produced by the human germ cell tumor line Tera-1 and originate mainly from a provirus on chromosome 22q11.21. *J. Virol.* 82, 10008–10016.
- Santarelli, R.L., Pierre, F., Corpet, D.E., 2008. Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence. *Nutr. Cancer* 60, 131–144.
- Sauerbrei, A., Wutzler, P., 2009. Testing thermal resistance of viruses. *Arch. Virol.* 154 (1), 115–119.
- Schlehofer, J.R., Ehrbar, M., zur Hausen, H., 1986. Vaccinia virus, herpes simplex virus, and carcinogens induce DNA amplification in a human cell line and support replication of a herpesvirus dependent parvovirus. *Virology* 152, 110–117.
- Schmitt, J., Schlehofer, J.R., Mergener, K., Gissmann, L., zur Hausen, H., 1989. Amplification of bovine papillomavirus DNA by N-methyl-N'-nitro-N-nitrosoguanidine, ultraviolet irradiation, or infection with herpes simplex virus. *Virology* 172, 73–81.
- Shuda, M., Feng, H., Kwun, H.J., Rosen, S.T., Gjoerup, O., Moore, P.S., Chang, Y., 2008. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc. Natl. Acad. Sci. U. S. A.* 105, 16272–16277.
- Small, M.B., Gluzman, Y., Ozer, H.L., 1982. Enhanced transformation of human fibroblasts by origin-defective simian virus 40. *Nature* 296, 671–672.
- Sutkowski, N., Chen, G., Calderon, G., Huber, B.T., 2004. Epstein-Barr virus latent membrane protein LMP-2A is sufficient for transactivation of the human endogenous retrovirus HERV-K18 superantigen. *J. Virol.* 78, 7852–7860.
- Taylor, E.F., Burley, V.J., Greenwood, D.C., Cade, J.E., 2007. Meat consumption and risk of breast cancer in the UK Women's Cohort Study. *Br. J. Cancer* 96, 1139–1146.
- Vajdic, C.M., McDonald, S.P., McCredie, M.R., van Leeuwen, M.T., Stewart, J.H., Law, M., Chapman, J.R., Webster, A.C., Kaldor, J.M., Grulich, A.E., 2006. Cancer incidence before and after kidney transplantation. *JAMA* 296, 2823–2831.
- Waibel, K.H., Walsh, D.S., 2006. Smallpox vaccination site complications. *Int. J. Dermatol.* 45, 684–688.
- Wang-Johanning, F., Radvanyi, L., Rycak, K., Plummer, J.B., Yan, P., Sastry, K.J., Piyathilake, C.J., Hunt, K.K., Johanning, G.L., 2008. Human endogenous retrovirus K triggers an antigen-specific immune response in breast cancer patients. *Cancer Res.* 68, 5869–5877.
- Yano, M., Wakabayashi, K., Tahira, T., Arakawa, N., Nagao, M., Sugimura, T., 1988. Presence of nitrosable mutagen precursors in cooked meat and fish. *Mutat. Res.* 202, 119–123.
- zur Hausen, H., 2001. Proliferation-inducing viruses in non-permissive systems as possible causes of human cancers. *Lancet* 357, 381–384.
- zur Hausen, H., 2006. *Infections Causing Human Cancers*. Wiley-VCH, Weinheim – New York, Publ.
- zur Hausen, H., 2009. Childhood leukemias and other hematopoietic malignancies: Interdependence between an infectious event and chromosomal modifications. *Int. J. Cancer* 125 (8), 1764–1770.
- zur Hausen, H., de Villiers, E.M., 2005. Virus target cell conditioning model to explain some epidemiologic characteristics of childhood leukemias and lymphomas. *Int. J. Cancer* 115 (1), 1–5 (May 20).