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Cancer causing viruses and the role of laboratory medicine: literature review and perspectives

Vírus causadores de câncer e o papel da medicina laboratorial: revisão de literatura e perspectivas

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ABSTRACT

Cancer-causing viruses are responsible for up to 20% of cancers with infectious etiology, representing a serious public health problem worldwide. Since the discovery of the first human cancer-causing virus, several others have been associated with neoplasias. Recent advances in technologies for the determination of genomic and proteomic profiles have resulted in the discovery and availability of tumor markers with potential application in the screening, diagnosis, prognosis and treatment of cancer. Therefore, laboratory medicine has stood out as a fundamental tool in the prevention and management of these diseases.

Key words: oncogenic viruses; tumor markers; laboratory test.

INTRODUCTION

Cancer is the main cause of mortality worldwide, mostly in developing countries, where it is responsible for approximately a fifth of total deaths[27]. Official estimates from the International Agency in Cancer Research indicate that up to 20% of total cancers are caused by infectious agents, mainly viruses[6]. This type of cancer represents a major public health problem in developing countries, low income populations and immunosuppressed patients from developed countries[24].

Discovery of cancer-causing viruses

In 1911, Peyton Rous concluded his experiments at the Rockfeller Institute in the USA, in which he transplanted a sarcoma from one hen into others from the same brood. That was the first evidence that cancer could be transmitted by cell-free extracts and that it could be caused by a small transmitting agent, probably a virus.

Similar experiments were published by two Danish scientists, Vilhelm Ellerman and Oluf Bang, who proved the viral transmission of avian erythroblastosis[13]. Notwithstanding, these evidences were regarded as scientific curiosities. Almost twenty years afterwards, new studies described the presence of virus in mammalian tumors in 1930[2].

The interest in the viral causes of cancer accrued in the early 1950's with the discovery of murine retrovirus and polyomavirus, which cause tumors in murines[20]. Only fifty three years after Rous's first experiments, the first human tumor-causing virus was described. The Epstein-Barr virus (EBV), also known as human herpesvirus 4 (HHV4), identified in 1964 by electronic microscopy of cellular lineages from African patients with Burkitt lymphoma[14].

Six other human cancer-causing viruses have been discovered in the last decades (**Table**), and others have been listed despite the fact that their role in human cancer has not been fully elucidated yet[24].

The discovery of hepatitis B virus (HBV) occurred after World War II when clinical and epidemiological studies started to differentiate several types of acute hepatitis. In 1967, Krugman *et al.* established the existence of at least two types of hepatitis, one of which presented parenteral transmission. It is known as serum hepatitis, currently called hepatitis B. Serological studies conducted by two independent

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TABLE – Human cancer-causing viruses

Virus	Genome	Cancer	Year	Reference
EBV, also known as HHV4	Herpesvirus with double-stranded DNA	Burkitt's lymphoma and nasopharyngeal carcinoma	1964	14
HBV	Hepadnavirus with partially double-stranded DNA	Hepatocellular carcinoma	1965	4
HTLV-1	Retrovirus with single-stranded RNA	Adult T cell leukemia	1980	29
HPV 16 and 18	Papillomavirus with double-stranded DNA	Cervical and penile cancer	1983-1984	5, 12
HCV	Flavivirus with single-stranded RNA	Hepatocellular carcinoma	1989	10
KSHV, also known as HHV8	Herpesvirus with double-stranded DNA	Kaposi sarcoma	1994	9
MCV	Polyomavirus with double-stranded DNA	Merkel cell carcinoma	2008	17

Adapted from Moore and Chang (2011)[24].
EBV: Epstein-Barr virus; HHV4: human herpesvirus 4; DNA: deoxyribonucleic acid; HBV: hepatitis B virus; HTLV-1: human T lymphotropic virus type 1; RNA: ribonucleic acid; HPV: human papillomavirus; HCV: hepatitis C virus; KSHV: Kaposi sarcoma herpesvirus; HHV8: human herpesvirus 8; MCV: Merkel cell polyomavirus.

research groups identified an antigen in the serum of patients with blood-borne hepatitis, namely the HBV surface antigen (HBsAg). These studies enabled the serological diagnosis of hepatitis B and initiated several epidemiological investigations[19]. The role of HBV in hepatocellular carcinoma was established a decade afterwards by means of longitudinal studies in areas of high HBV prevalence[1, 3].

In 1980, the human T lymphotropic virus type 1 was discovered and identified by culture and electronic microscopy of adult T cell lymphoma. It was the first human retrovirus described as a potential cause of malignity[18, 29]. Some time afterwards the high risk human papillomaviruses ([HPV] 16 and 18) were described as the main causes of cervical cancer by means of hybridization of papillomavirus deoxyribonucleic acid (DNA). In studies that investigated the role of this virus in sexually transmitted genital warts, they were known as cervical cancer DNA[5, 12].

The causing agent of blood-borne hepatitis continued to be investigated until the end of 1980's. In 1989, Qui-Lim Choo *et al.* isolated hepatitis C virus (HCV) in serum from a patient with hepatitis non A non B in the USA. This virus (HCV) was immediately associated with hepatocellular carcinoma as well as HBV[10].

More recently, the Kaposi sarcoma herpesvirus (KSHV), also known as human herpesvirus 8 (HHV8) and the Merkel cell polyomavirus (MCV) have been discovered, both through experiments based on nucleic acid extraction[9, 17].

The events from this first century of investigations into tumoral infections caused by virus culminated with the Nobel prizes in 2008 for the discovery of high risk HPV strains by Harald zur Hausen and the discovery of the acquired immunodeficiency virus (HIV) by Fraçois Barré-Sinoussi and Luc Montagnier. The former are responsible for cervical cancer and the latter is an agent that does not initiate cancer, yet it indirectly fosters malignity by means of immunosuppression[24].

Mechanisms of viral oncogenesis

Cancer is the final result of several genetic cellular changes, which may be responsible for the alteration in the balance of some mechanism such proliferation, programmed cell death (apoptosis) and cell transformation. The neoplastic cell transformation may be divided into initiation, promotion and progression. Furthermore, there is generally a long latent period from the moment of carcinogenic exposure to the neoplastic transformation, in which somatic cells are capable of proliferating without appropriate control, accumulating multiple genetic mutations that ultimately may result in disease[8].

The oncogenesis mechanisms in humans are commonly multifactorial and complex, inasmuch as several factors may act on different mechanisms and in different stages of tumoral development. Thus, it is difficult to pinpoint which mechanism a carcinogen is acting on, although its association with a specific type of cancer has been established by epidemiological data.

According to Schulz[32], despite its diversity, human cancers exhibit fundamental properties that may aid to grasp the mechanisms of viral oncogenesis:

- augmented cell proliferation;
- insufficient apoptosis;
- altered cell and tissue differentiation;
- altered metabolism;
- genomic instability;
- immortalization (growth in spite of replicative senescence);
- invasion of different tissue layers;
- metastasis in local lymph nodes and distant tissues.

Some of them are discussed herein.

The viral infection of a cell may result in the alteration of its properties, as it occurs in the so called cellular transformation, which

generally includes the loss of growth control, anchorage-independent growth, ability to invade extracellular matrix, loss of differentiation and immortalization. Transformed cells commonly present chromosomal abnormalities, which may result in the integration of viral genome into hostage cell chromosomes.

Cancer cell hyperproliferation is a result of an altered response to controlling signals of cellular growth. These cells are hypersensitive to growth stimulus signals, frequently becoming independent of them. Furthermore, the sensitivity to growth inhibitory signals is diminished or even absent.

Therefore, there are two mutational routes by which cellular proliferation becomes uncontrolled, which may characterize cancer. The first occurs when a "stimulator" gene is hyperactive (oncogene) and the second when an "inhibitor" gene is inactive (tumor suppressor gene)[32].

The viral genome region that is able to generate a tumor is known as oncogene. This gene may be incorporated into a hostage cell by the virus, resulting in alteration of its properties[25].

The first group of oncoges discovered by the end of 1970's belonged to the genome of acute transforming retroviruses, which cause cancers in birds and mammals. The second group of oncogenes consists of hostage proto-oncogenes, which become active when the insertion of a slow transforming retrovirus disturbs its regulation[23].

Nevertheless, retroviral insertion is only one among several mechanisms capable of prompting cellular pro-oncogenes to act as oncogenes. Other mechanisms include chromosomal translocation, genetic amplification and punctual mutation. They change the regulation and/or function of cellular genes, which are then activated, hence becoming oncogenes. Consequently, their proteic products may become overexpressed, deregulated, hyperactive or poorly located in the cell[32].

Several cellular proto-oncogenes regulate cellular proliferation, differentiation and survival also in its normal state. Some of them act as extracellular growth factors, others as their receptors and some of them as juxtamembrane adaptors or transducers in signaling cascades. Kinase proteins are another significant type of proto-oncogene products[16].

There are a few cases in which only one oncogene is sufficient to generate malignity. In fact, only one oncogene provides some aspects of malignant phenotype and cooperates with others or with defects in tumor suppressor genes in the complete transformation[16].

Whereas oncogenes promote the development of tumors due to the increase in its activity or deregulation, the suppressor genes need to lose its function so that the tumor occurs.

Retinoblastoma represents a paradigm for tumor suppressor genes, inasmuch as the inactive tumor suppressor gene of this disease (RB1) codifies a central regulator of cellular cycle, predominantly G1→S transition, which apart from ensuring the accurate mitotic segregation, it affects chromatin structure and regulates apoptosis[32].

RB1 product (protein pRB) acts by means of binding and control of other proteins, mainly E2F transcriptional factors, which have the capacity to activate the required genes for the entrance in the S phase cell cycle and for DNA synthesis. In some cases, it may induce apoptosis. pRB is regulated via phosphorylation by cyclin-dependent kinases, which are linked with their own regulatory subunits[38].

On the one hand, the loss of pRB function directly implies cellular proliferation and differentiation. On the other hand, the loss of suppressor genes such as TP53 compromises the cell ability to react to damages in the genome, which may occur in viral infections. Accordingly, the function loss of this gene allows the survival and cellular proliferation with an accumulation of mutations, hence promoting cancer[22].

Protein p53 plays a pivotal role in the regulation of several signaling pathways that control cellular response to DNA damages caused by different agents such as ultraviolet light, ionizing radiation and chemical carcinogens. It is a transcriptional regulatory protein induced by the response to DNA damage. Furthermore, it may lead to the stop of cellular cycle or induce apoptosis in response to metabolic depletion, thermal shock, hypoxia, viral oncoproteins and activated cellular oncogenes. In these situations, p53 is stabilized, it accumulates rapidly, undergoes post-translational modifications and it regulates the progression of cellular cycle at checkpoinst so that DNA may be repaired. In case the damage persists, the cell is eliminated by apoptosis[34].

Apoptosis plays a major role in the regulation of normal cell population. The suppression of this process may be a decisive factor in cancer development. The inability to respond to DNA damage, either with the cell cycle arrest or apoptosis induction, results in one of the aspects of cancer cells: genomic instability[28].

This genomic instability may contribute to a rapid selection of cellular populations capable of overcoming environmental factors that arise during the tumoral progression. Accordingly, the genomic instability has been described as a major characteristic in tumoral cells[21].

The role of laboratory medicine

Currently, the investigation into tumoral markers is one of the fastest growing areas in laboratory medicine[11]. Tumoral markers are molecules that present biological alterations in cellular functions commonly associated with malignity, which may be detected in the tumoral tissue, serum, plasma or in other body fluids. These substances are generally proteins or glycoproteins, though phospholipids, DNA or ribonucleic acid (RNA) may also be directly produced by the tumor or in response to tumor presence[33].

Similar to other diagnostic tests, tumoral markers are cancer surrogate markers, with potential clinical application in the

assessment of risk, early detection, diagnosis, molecular classification, prognosis, as well as evaluation of application and response to treatment, metastasis and recurrence[35].

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer worldwide and the third most common cause of cancer-related, with over 500 thousand cases diagnosed annually. The incidence of HCC is highly variable, depending on geographic region and exposure to risk factors. 70%-90% of patients with HCC present history of chronic hepatopathy and cirrhosis, whose main risk factors include infection by HBV, HCV, alcoholic hepatic disease and non-alcoholic hepatic steatosis. Considered one of the most aggressive forms of cancer, HCC generally presents bad prognosis due to late diagnosis, advanced staging, mostly untreatable[15].

For the risk population, the active tracking of HCC is recommended through abdominal ultrasonography and periodic evaluation of tumoral markers. In this specific case, the rise in serum alpha-fetoprotein levels (AFP) may be the first indication of malignity, which must be investigated through imagery test, including CT scan and magnetic resonance imaging, as well as ultrasonography or histological exams through biopsy.

Although several HCC tumoral markers are currently under investigation, AFP is the only hepatic cancer marker applied in the clinical practice. AFP is a glycoprotein 70kD, normally produced during gestation, and may be assessed by immunoassays with monoclonal and/or polyclonal antibodies. AFP should not be considered a diagnostic marker in isolation due to its low sensitivity and specificity. Approximately 40% of patients with HCC present normal levels of AFP and high levels of it may be observed in patients with chronic hepatopathy or cirrhosis, in the absence of HCC and in patients with other malignities. The determination of AFP has been recently removed from the recommendations endorsed by the American Association for the Study of Liver Diseases (AASLD)[7].

Cervical cancer

Cervical cancer is the main cause of death by gynecological cancer worldwide, with high incidence mostly in developing countries. High risk HPV (types 16, 18, 45 and 56) are the main agents involved in the pathogenesis of cervical cancer[37].

The screening in asymptomatic women through Papanicolaou smear allows the diagnosis of treatable pre-invasive lesions, thus avoiding the progression to malignity. Nevertheless, in developed countries most cases of cervical cancer occur among women that did not undergo regular screening. On the other hand, in developing countries, the access to these services are not available and most women present an advanced stage of the disease, with involvement of the bladder, rectum, pelvic nerves and bones[37].

Approximately 85% of the cases are squamous cell cervical carcinomas. The other histological types include adenocarcinoma (10%-15%) and adenosquamous carcinoma (3%)[37].

The tumoral markers may aid in the approach to cervical cancer and several other potential markers have been researched. The choice marker for squamous cell carcinoma is the squamous cell carcinoma antigen (SCC), whose serum levels are correlated with staging, tumor size, residual presence after treatment, recurrence, disease progression and survival. However, it is not sufficiently sensitive or specific to screening and diagnostic purposes[36].

SCC is a subfraction of TA-4, an antigen associated with tumors, which belongs to serine-protease inhibitors. Studies on molecular cloning in genomic regions have revealed the presence of two genes that codify SCC in two isoforms. The neutral isoform is present in tissue and epithelial cells regardless of malignity, whereas the acidic isoform is found exclusively in tumor cells, mainly those located in the tumor peripheral area and serum from patients with well differentiated squamous cell carcinoma[26].

High levels of SCC are found in the initial diagnosis of approximately 60% of patients with cervical cancer. More specifically, serum SCC is high in approximately 24%-53% of patients in the initial stage and 75%-90% in advanced stages[26].

The carcioembryonic antigen (CEA) and CA125 may be useful in patients with cervical adenocarcinoma.

PERSPECTIVES

As it commonly occurs in any branch of medicine and clinical research, the development of genomic and proteomic technologies have changed the approaches and experimental applications of tumor markers. Initiating with genetic and molecular biology technologies used in the analysis of the human genome, the recent years have been characterized by the growing use of techniques for the determination of genomic and proteomic profiles such as the following: oligonucleotids and complementary DNA microarrays, array-based comparative genomic hybridization (CGH), serial analysis of gene expression (SAGE), two-dimensional electrophoresis in polyacrylamide gel (2-DPAGE), mass spectrometry with matrix assisted laser desorption ionization (MALDI), surface enhanced laser desorption ionization (SELDI) and time of flight (TOF), among others[33]. These technologies combined with clinical interpretation of molecular diagnosis and molecular medicine have accelerated advances in information and technology in this research area[33]. These changes will certainly affect the field of tumor markers and their application in the near future, resulting in better diagnostic tools, prognosis and cancer treatment. Therefore, laboratory medicine will play an increasingly outstanding and fundamental role in this area.

RESUMO

Os vírus causadores de câncer são responsáveis por até 20% dos cânceres de etiologia infecciosa, representando um grave problema de saúde pública em todo o mundo. Desde a descoberta do primeiro vírus causador de neoplasias em humanos, vários outros têm sido associados ao câncer. Recentes avanços nas tecnologias de determinação de perfis genômicos e proteômicos resultaram na descoberta e na disponibilização de marcadores tumorais com potencial aplicação no rastreamento, no diagnóstico, no prognóstico e no tratamento do câncer, destacando a medicina laboratorial como ferramenta fundamental na prevenção e no manejo dessas enfermidades.

Unitermos: *vírus oncogênicos; marcadores de tumor; testes laboratoriais.*

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