Molecular biology of tumor progression

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In the past 15 years we have accumulated a great deal of knowledge about the pathogenesis of tumors at the molecular level. The majority of work has defined and characterized the genetic alterations present in tumor cells, but little light has so far been shed on the process of carcinogenesis.

If we consider cancer a genetic disease of the cell and carcinogenesis a process that requires the accumulation of mutations, the process of the 'cancerization" of a tissue and the emergence of a tumor can be regarded as a microevolutionary process. For the common epithelial malignancies in the human, the time period necessary for the emergence of a symptomatic tumor ranges from 10-20 years. Studies of preneoplastic tissues at risk for developing tumors show that: i) the rate of mutation is not significantly increased; ii) a diversity of mutations can be demonstrated to coexist in different micro-clones and iii) carcinogenic agents tend to reduce the diversity present in preneoplastic tissues.

Some promoters can be regarded as selective forces that will ultimately be responsible for the specific combinations of mutations found in tumor specimens. Molecular epidemiology studies in humans and studies in animal models have suggested a number of environmental factors (promoters) that act as selective forces and, in some instances, we have begun to unravel the mechanisms by which selection occurs. Selection shapes the genotype of tumors by playing on a checkerboard of mutations. The technologies required for microevolutionary studies of somatic cell populations and some recent results from our laboratory will be reviewed.

Molecular detection of micrometastases: Its impact in the assessment of prognosis

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The most important pathological parameter of poor prognosis in cancer patients with solid tumors is the presence of metastatic diffusion to lymph nodes. However, a consistent portion of patients with pathologically negative lymph nodes ultimately develop recurrent cancer, suggesting that current methods are inadequate for identifying metastatic disease. The standard histopathological examination of tissue sections, used routinely for the detection of metastatic tumor cells, has the sensitivity to find one abnormal cell in a background of about 19~ normal cells. However, the main limiting factor is the number of sections made and examined. Cutting one or a few sections from the center of the node, samples about 1/1,000 of the tissue submitted for pathological examination. Serial sectioning and/or immunohistochemistry of lymph nodes can increase detection of metastatic cells by 10-30% over that obtained by standard sectioning with hematoxylin and eosin staining. It has been suggested retrospectively that patients with occult metastases detected by serial sectioning or immunohistochemistry may have a worse prognosis (1, 2). Sectioning and staining, however, is too cumbersome and costly to be routinely performed.

Technical advances now permit the detection of metastatic disease at the molecular level. For example, somatic mutations in oncogenes or tumor-suppressor genes that occur in primary tumors are also detectable in lymph node DNA. Detection of micrometastases by this method has been attempted in colon and pancreatic cancer and could be performed in lung cancer patients who frequently show p53 and *K-ras* mutations in their primary tumors (3-7). This approach, however, is not possible in tumor types, such as breast or prostatic cancer, that lack frequent mutations suitable for amplifications by polymerase chain reaction (PCR). A more general approach is the possibility of amplifying cancer cell-specific RNA messages from lymph nodes by the reverse-transcriptase POR (RT-PCR) assay. This latter method has been used to detect micrometastases in lymph nodes from patients affected by the most common forms of human solid tumors, including carcinomas of the breast, colon, lung and prostate as well as melanoma (8-12). The ideal messenger RNA marker, expressed at high levels in tumor tissues and not at all in normal lymph nodes, remains to be identified. However, various markers, sometimes multiple markers at a time, have successfully been used with RT-PCR to detect lymph node metastases. For some tumors, including prostatic cancer, melanoma and breast carcinoma, tissue-specific RNA markers are now available; for other tumors, such us colon and lung carcinomas, more general, tumor-specific markers have to be used.

It has been reported that when molecular methods are used about 30% of breast carcinomas, 40% of colon carcinomas, 35% of lung carcinomas, 45% of prostatic carcinomas and 45% of melanomas show micrometastases not detectable by routine histology.

The high sensitivity of molecular methods for the detection of micrometastases makes them particularly suitable for the screening of metastatic diffusion in sentinel lymph nodes, It has recently been reported both in patients with breast cancer and with melanoma that the RT-PCR assay offers improved detection of occult metastases in the sentinel node over conventional hematoxylin-eosin and immunohistochemical staining (13-14).

The presence of micrometastases in lymph nodes detected by molecular methods correlates with pathological and biological parameters of poor prognosis in carcinomas of the breast and colon, as well as in melanomas (3, 13, 15). Recently, a clear-cut correlation between the presence of node micrometastases, evaluated by RT-PCR and survival of stage II colorectal cancer patients, has been observed (9).

These findings suggest that molecular analysis of resected lymph nodes in cancer patients could lead to a more accurate prognosis and to a better selection of patients who could benefit from adjuvant therapy. Long-term studies of PCR-positive patients are required to better understand its overall clinical relevance and predictive value.

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Viral genes in human tumors

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The development of malignant human tumors is a complex and slow process that requires the accumulation of multiple genetic alterations. The activation of oncogenes and the inactivation of tumor suppressor genes are necessary and the inhibition of apoptosis seems to be an important step in most tumors, at least in the early stages of malignant transformation. The genetic alterations include mutations, deletions and gene amplifications and, in many cases, a background of genetic instability.

There are many carcinogenic agents, such as chemical compounds, -y-irradiations and viruses. Viruses constitute the second most important etiological factor in human tumors and about 10% of malignant tumors are associated with them. Retroviruses are the most frequent; amongst them are the lymphotropic HTLV, the Moloney retrovirus and so on. Retroviruses may induce insertion mutagenesis and can drive activated oncogenes into the cells. DNA viruses are also numerous and several important groups can be recognized: human papilloma viruses, with over 70 serotypes, which can infect most epithelia and are associated with cervical, skin and upper airway carcinomas; hepatitis B and hepatocarcinomas; Epstein-Barr virus and lymphomas, nasopharyngeal carcinomas, etc.; SV4O virus and some mesotheliomas and brain tumors; herpes type 1 with stromal tumors; herpes type 2 and cervix carcinomas; herpes type 8 with Kaposis sarcoma and body cavity lymphomas.

Viral genes may interact with the main pathways involved in carcinogenesis. In fact, some of their products are capable of inducing cell proliferation, binding tumor suppressor proteins and inhibiting them and activating or inhibiting gene products related with apoptosis. Some viral genes are associated with genetic instability. For example, herpes type 1 may induce microsatellite instability while human papilloma virus and adenoviruses can provoke chromosome instability. Although the association between viruses and cancer is clear in many human tumors, it has to be stressed that, in addition to the viral effects, the accumulation in the cell of other genetic alterations is necessary for the malignant transformation to occur.

On the other hand, viruses play a relevant role in the treatment of human tumors. They are the best vectors to deliver therapeutic genes and most gene therapy protocols are based on viruses. Both retroviruses and adenoviruses are capable of transferring tumor suppressor genes, suicide genes or genes related to apoptosis. There are many clinical approaches in transducing p53, p16, pRb genes or the adenoviral EIA gene. Finally, it is interesting to know that some adenoviruses can replicate more efficiently in malignant cells with selective genetic alterations. These defective adenoviruses proliferate in cells with oncogenic alterations, such as a mutated p53 gene, and kill them; this represents one of the most promising areas in cancer gene therapy.

In this course, we will study the viruses and viral genes most frequently involved in human tumors, analyze the cellular pathways that are activated or inactivated by viral genes and, finally, discuss the perspectives of cancer gene therapy based on viruses.

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