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AIDS-MALIGNANCIES AND SIGNALING NETWORKS: A CASE OF MOLECULAR HIJACKING

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Kaposi's sarcoma (KS) is the most common cancer arising in patients with acquired immunodeficiency syndrome (AIDS). The recent introduction of highly active antiretroviral therapy (HAART) has witnessed a dramatic decrease in the proportion of new AIDS-defining KS cases as well as a regression in the size of existing KS lesions. However, there is still a risk of recurrence of AIDS-associated KS that we cannot afford to ignore. Indeed, in parts of the developing world, KS has tragically emerged as one of the most frequent cancers among children and adult men, and KS remains a significant cause of morbidity and mortality among the world AIDS population. The recent identification of the Kaposi's sarcoma herpesvirus (KSHV) as the etiologic agent for KS promised a new era in KS research. However, characterization of the KSHV genome revealed numerous potential oncogenes whose relative contribution to KS development and their interplay with HIV genes and/or immunosupression still remains poorly understood. To begin addressing this issue, we have recently developed a high throughput *in vivo* endothelial specific retroviral gene transfer system, and used it to express candidate KSHV oncogenes in mice. Surprisingly, among the many KSHV genes tested, only one gene, a constitutively active G protein-coupled receptor, *vGPCR*, was able to promote the development of visible vascular tumors that strikingly resembled human KS lesions. Furthermore, we provided evidence that *vGPCR* can transform endothelial cells as well as promote the tumoral growth of cells expressing KSHV latent genes in a paracrine fashion. Thus, *vGPCR* may play a critical role in initiating KS tumor development, and can also unmask the sarcomagenic potential of KSHV latent genes. Recent progress in the study of the molecular mechanisms underlying the transforming activity of *vGPCR* will be presented. Ultimately, this knowledge may contribute to the identification of novel strategies to treat and prevent this devastating disease.

THE ANGIOTENSIN AND ENDOTHELIN-CONVERTING ENZYMES: MOLECULAR TARGETS IN VIROLOGY, CANCER AND CARDIOVASCULAR SCIENCES

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The angiotensin and endothelin-converting enzymes constitute two families of zinc metalloproteases that have diverse functions in human physiology and pathology and constitute important therapeutic targets. They are ectoenzymes, i.e. proteins anchored in the plasma membrane with their catalytic sites exposed to the external surface of the membrane. These proteases are widely expressed, and their dysregulated expression is associated with cancer, infection, inflammation, autoimmune and cardiovascular diseases. Angiotensin converting enzyme (ACE) has only one homologue in the human genome, termed ACE2, and these two enzymes act as counterbalances in the renin-angiotensin system through their production of angiotensin II and angiotensin-(1-7) respectively. Hence, dysbalance of these activities can lead to cardiovascular complications, as well as angiogenesis. Genetic factors related to ACE may also influence the behaviour of human prostate cancer. Serendipitously, ACE2 turns out to be the receptor for the severe acute respiratory syndrome (SARS) coronavirus and mediates viral binding to the surface of epithelial cells and internalization. The endothelin-converting enzymes (ECE-1 and ECE-2) catalyse the final step in the biosynthesis of the potent vasoconstrictor

and mitogenic endothelin peptides. They are closely related in structure to neprilysin (NEP), which inactivates the endothelins and other mitogenic peptides. Again, dysbalance of the levels of NEP and ECE can contribute to cardiovascular disease and cancers. For example, NEP is dramatically down-regulated in prostate cancer allowing the progression of androgen-independent disease. NEP and ECE are reciprocally regulated and stromal-epithelial interactions influence prostate cancer cell invasion by altering the relative balance of their expression. The endothelin system may play a pivotal role in other cancers, e.g. squamous cell carcinoma, where we have shown that siRNA targeting of ECE-1 can block cell proliferation. The biochemistry, cell biology and pathology of these two enzyme families will be compared and their status as therapeutic targets evaluated.

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GENE ALTERATIONS IN THYROID CANCER: ROLE OF THE RECEPTOR TYROSINE PHOSPHATASE DEP-1/*PTPRJ*, IN THYROID CARCINOGENESIS

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Thyroid tumors include a wide spectrum of lesions with different phenotypic characteristics and biological behaviour: benign adenomas, differentiated carcinomas and anaplastic carcinomas.

At the moment, the involvement of several oncogenes has been demonstrated in papillary thyroid carcinomas. Activation of the RET/PTC oncogene, caused by rearrangements of the RET protooncogene, has represented for years the main genetic alteration in papillary carcinomas since it is detectable in 40% of the cases. However, more recently, mutations of the B-raf gene have been demonstrated in almost 50% of papillary carcinomas. TRK gene rearrangements and MET gene overexpression are often found in carcinomas of the papillary type. Conversely, *ras* gene mutations and PAX8-PPAR-1 rearrangements are frequently detected in tumors of the follicular type. Impairment of the p53 protein function represents a typical feature of the anaplastic carcinomas.

These genetic lesions have a critical role in the process of thyroid carcinogenesis as demonstrated by the transformation of thyroid cells by these oncogenes and by the development of papillary thyroid carcinomas in mice carrying these oncogenes under the transcriptional control of thyroid promoter.

However, it is known that the carcinogenesis is a multistep process that requires more genetic lesions in the same cells. Because of this, it is important the identification of other genes as tumor suppressor genes involved in thyroid carcinogenesis.

To this purpose, we recently isolated a candidate tumor suppressor gene for thyroid carcinogenesis. It is the rat tyrosine phosphatase ? (*r-PTP??*?) gene. It codes for a receptor type phosphatase composed of an extracellular region containing eight type III fibronectin repeats, a transmembrane region and an intracellular region with a single catalytic domain. Suppression of the *r-PTP?* gene expression has been demonstrated in retrovirally infected cells showing a highly malignant phenotype, such as the rat thyroid PC Cl3 and FRTL5 cells which are transformed by the myeloproliferative sarcoma virus and by the Kirsten murine sarcoma virus, respectively. Subsequently, a drastic reduction of the HPTP??DEP-1 protein, the human homologous of r-PTP?, has been shown in human thyroid carcinomas. Moreover, restoration of r-PTP? expression in malignant rat thyroid cells and in human thyroid carcinoma cell lines inhibits their growth and tumorigenicity. Cell cycle analysis of r-PTP? transfected cells demonstrated that r-PTP? caused G1 growth arrest and increased the cyclin-dependent kinase inhibitor $p27^{Kip1}$ protein level by reducing the proteasome-dependent degradation rate. We proposed that the r-PTP? tumor suppressor activity is mediated by $p27^{Kip1}$ protein stabilization, because suppression of $p27^{Kip1}$ protein synthesis using p27 specific antisense oligonucleotides blocked the growth inhibitory effect induced by r-PTP???Furthermore, we provided evidence that in *v-mos*- or *v-ras-Ki*-transformed thyroid cells, $p27^{Kip1}$ protein level was regulated by the MAP kinase pathway and that r-PTP? regulated $p27^{Kip1}$ stability by preventing *v-mos*- or *v-ras-Ki*-einduced MAP kinase activation.

On the basis of these results, we took in consideration a cancer gene therapy of human thyroid carcinomas

based on the restoration of PTP?/DEP-1 expression, and, therefore, we generated an adenovirus carrying the full-length r-PTP? cDNA (Ad-PTP?). We showed that the Ad-PTP? virus is able to inhibit thyroid carcinoma cell growth. The growth inhibition effect of the Ad-PTP? virus was associated with an increased p27 protein level, a reduced MAPK activity and a reduced tyrosine phosphorylation level of PLC?1, a known substrate of DEP-1. Finally, the growth of xenograft tumours induced in athymic mice by the injection of ARO cells was drastically reduced by the Ad-PTP? treatment.

We searched for the PTP?-interacting proteins, in order to elucidate the mechanisms by which the tyrosine phosphatase exerts its action. We found that r-PTP? protein binds to c-Src in living cells. We also reported that r-PTP? dephosphorylates the c-Src inhibitory tyrosine phosphorylation site (Tyr 529) thereby increasing the c-Src tyrosine kinase activity in malignant rat thyroid cells, stably transfected with r-PTP?. Enhanced tyrosine phosphorylation of FAK and paxillin was observed in r-PTP??expressing cells. It appears associated with an increased adhesion of the r-PTP??transfected transformed thyroid cells in comparison with the corresponding untransfected cells or those stably transfected with the inactive mutant form of r-PTP?? The treatment of the thyroid infected cells with the c-Src inhibitor PP2 decreases cell adhesion at higher extent in the r-PTP??transfected cells indicating that r-PTP? positively regulates cell-substratum adhesion by c-Src activation. Interestingly, the extent of c-Src dephosphorylation at Tyr 529, FAK and paxillin phosphorylation and the increase in cell adhesion are dependent on r-PTP??expression levels.

Recently, it has been shown that loss of heterozygosity and missense mutations of this gene are frequent in human colon, lung and breast cancers. Furthermore, the mouse homolog of *r-PTP?*/*DEP-1*, *Ptprj*, is the candidate gene for the mouse colon cancer susceptibility locus Scc1. We performed loss of heterozygosity (LOH) analysis for *PTPRJ* in a panel of human thyroid tumors. We detected loss of heterozygosity (LOH) of *PTPRJ* in about 15% of informative thyroid tumors. In the same panel and in a control group of healthy individuals, we performed sequence analysis of exons 5, 6 and 13 to detect Gln276Pro, Arg326Gln and Asp783Glu polymorphisms. We found that the *PTPRJ* genotypes homozigous for the Gln276Pro and Arg326Gln polymorphisms and the Asp873 allele were significantly more frequent in thyroid carcinoma patients than in healthy individuals. These results indicate that the genotype profile of *PTPRJ* affects susceptibility to thyroid carcinomas, and that allelic loss of this gene is involved in thyroid carcinogenesis.