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Specific and redundant functions of mismatch repair proteins in mutation avoidance and suppression of cancer

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Cancer predisposition in HNPCC, the non-polyposis form of familial colon cancer, is caused by defects in post-replicative DNA mismatch repair (MMR). This system recognizes and restores mismatched nucleotides that can arise by erroneous DNA replication, bypass replication of damaged DNA, incorperation of modified nucleotides or in heteroduplex regions that are formed during homologous recombination between non-identical DNA molecules. We have recapitulated HNPCC in mice by disrupting the central MMR genes Msh2, Msh6 and Msh3. The gene products form heterodimeric protein complexes with specific and redundant mismatch recognition capacity. Mice carrying a homozygous disruption in Msh2 or Msh6 were equally sensitive to development of lymphomas and epithelial tumors of the skin and uterus. However, in contrast to Msh2-deficient animals, Msh6-deficient mice only rarely developed intestinal tumors. The latter tumor type did develop upon combined Msh3/Msh6 deficiency. These results indicate that some tumor types (lymphoid, uterine, skin tumors) ensue from mutations that are specifically suppressed by MSH6 function, while intestinal carcinogenesis requires genetic alterations that are suppressed by both MSH6 and MSH3. These observations in mice are consistent with the rarity of MSH6 and lack of MSH3 germ line mutations in classical HNPCC families. Our present aim is to identify the specific mutation avoidance functions of MSH6 and MSH3. In addition to cell lines with complete ablation of mismatch recognition proteins, we have constructed a cell line with a tenfold reduced MSH2 protein level. Remarkably, these cells had retained almost maximal mismatch-repair capacity, but were as resistant to cell killing by methylating agents as fully Msh2-deficient cells. Importantly, in contrast to wild-type cells, MNNG-induced mutagenesis in these cells was almost a high as in MSH2-deficient cells. Thus, low levels of MSH2 renders cells resistant to the toxic, but highly sensitive to the mutagenic effects of methylating agents. These observations may have implications for both the etiology and treatment of cancer.

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Anti-angiogenic approaches for cancer gene therapy

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The anti-tumoral effects which follow the delivery of genes coding for anti-angiogenic activities, such as angiostatin or ATF, are studied using different tumor models.

Angiostatin, a physiopathological inhibitor of angiogenesis secreted by primary tumors, drives the metastasis into a dormant state. We have already shown that a single intratumoral injection of a recombinant adenovirus coding for this factor (amino-acids 1-333 of plasminogen) can inhibit growth of different types of tumors as a consequence of a decreased vascularization. In order to increase the half-life of the molecule in vivo, we have fused it to HSA (human serum albumin) and studied the biological properties of the fused molecule in cell culture and in vivo. We show that the fusion does not modify the activity on endothelial cells in culture and that the systemic expression of the fused molecule efficiently inhibits growth of grafted tumors. The IV injection of the virus was also found to block metastasis dissemination in mice which develop tumors of the retinal pigment epithelium. We have also compared the efficacy of gene expression following adenoviral vector delivery and plasmid electrotransfer. The level of circulating angiostatin was found to be ten times more elevated after adenoviral delivery than after plasmid electrotransfer; nevertheless, the expression was much more durable in this latter case. The antitumor response following electrotransfer is in progress.

We have also assessed in mice the antitumoral effects which follow the administration of a recombinant adenovirus coding either for human ATF or for an HSA-fused human ATF. ATF is the amino-terminal end of urokinase and acts as a soluble antagonist of urokinase binding to its receptor. We show that fusion of ATF to HSA increases the half-life of the molecule in vivo; thus, the seric concentration of ATF-HSA is 500-1000 fold more elevated than the one of ATF and this without altering the activity of ATF. Whereas the intratumoral delivery could inhibit growth of grafted tumors, no effect was observed following a systemic administration of the virus.

Our results suggest that electrotransfer of plasmids coding for HSA-fused angiostatic factors would permit a sustained and high level expression of angiostatic factors, a prerequisite for maximal clinical benefits.

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Models of angiogenesis for evaluation of anti-angiogenic drug mechanisms

Abstract not received.

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Target-specific anti-angiogenic cancer therapy

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The growth of solid tumors is critically dependent on the de novo establishment of a vascular system. This process involves the secretion of angiogenic factors by the tumor and corresponding receptors on endothelial cells. Previously we have identified the receptor tyrosine kinase Flk-1/KDR (VEGFR2) as a receptor for vascular endothelial growth factor (VEGF) and demonstrated that angiogenesis in a variety of tumor types is absolutely dependent on cellular signals generated by the VEGF/VEGFR2 system. In nude mouse xenograft models inhibition of the VEGFR2 signaling function results in tumor growth inhibition and tissue necrosis. Based on these findings inhibitors of the VEGFR2 signaling activity were developed by a number of pharma companies. Inhibitors of the receptor kinase function are currently being tested in phase I and II clinical trials for their efficacy in the treatment of a variety of solid tumors. Results of preclinical studies and clinical trials will be presented.

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Tyrosine kinase receptors as target for anti-angiogenic approaches

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Tumor-induced angiogenesis is mainly sustained by the production and secretion of angiogenic factors originating from tumor and stroma cells. The most prominent angiogenic factor is the vascular endothelial growth factor VEGF. Recently, additional angiogenic factors and their respective receptors have been identified and related to tumor angiogenesis. Among these, the angiopoietins and their receptor TIE-2 have been investigated to some detail. Angiopoietin-1, which binds to and activates TIE-2, is obviously responsible for the stabilization of vessels under homeostatic conditions. Angiopoietin-2 binds to the same receptor as angiopoietin-1 but is antagonistic with respect to angiopoietin-1. It destabilizes blood vessels and under appropriate conditions induces complete regression. When human melanoma cells A375 are stably transfected to produce the soluble variant of the angiopoietin receptor TIE-2 (sTIE-2) they show a substantial inhibition of tumor growth on nude mice. Similar effects have been seen with the soluble variant of the VEGF-receptor FLT-1 (sFLT-1). In both cases, the vessel density of the tumors is significantly reduced. Thus, inhibition of both signalling systems seem to be a valid strategy for the development of novel anti-angiogenic therapies. Recently, the inhibition of the VEGF-receptor tyrosine kinase by the compound PTK787/ZK222594 has been shown to substantially inhibit tumor growth and metastases formation, this compound has now entered clinical trials at the Tumor Biology Center in Freiburg. A preliminary evaluation of phase I will be presented.