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Role of plasminogen activation system

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Cancer invasion can be considered as a tissue remodelling process in which one tissue, the normal tissue, is substituted by another, the invading cancer tissue. Studies of human cancer show that during invasion, extracellular proteases (e.g. uPA or an MMP), their inhibitors (e.g. a PAI or a TIMP) and their receptors (e.g. uPAR) are often expressed by tumor infiltrating stromal cells, such as fibroblasts, endothelial cells, macrophages and neutrophils. This suggests that generation and regulation of extracellular proteolysis in cancer invasion, like in many non-neoplastic tissue remodelling processes, is accomplished by an interaction between several cell types. The pattern of expression of components of protease systems appears to be unique for each type of cancer. The expression in cancer often mimics that in certain non-neoplastic tissue remodelling processes. Examples are ductal breast cancer mimicking postlactational mammary gland involution and squamous cell skin carcinoma mimicking skin wound healing. Prognostic as well as experimental studies support that the stromal cell produced components of protease systems have an important function in cancer progression. We propose that cancer invasion can be considered as specific non-neoplastic tissue remodelling processes gone out of control. This is in analogy with neoplastic growth being considered as normal growth gone out of control. A crucial difference naturally being that non-neoplastic tissue remodelling stops while cancer invasion does not. This approach implies that an understanding of the basic mechanisms involved in non-neoplastic tissue remodelling is crucial for an understanding of cancer invasion. This will be illustrated by studies of cancer invasion and metastasis as well as non-neoplastic tissue remodelling in mice deficient for genes encoding components of the plasminogen activation system. The therapeutic implications of these studies will be discussed. The stromal cell involvement has profound consequences for understanding of both carcinogenesis and establishment of metastasis. It is thus not enough for cancer to arise, that a cell by mutation acquires the characteristics of a cancer cell. It is also necessary that it recruit the right combination of stromal cells. Likewise it is not enough for establishment of a metastasis, that a cancer cell settles in a new tissue. In order to develop a metastasis, it is also necessary that it recruit the right combination of stromal cells.

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Stroma activation and angiogenesis in tumour progression

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Interactions of tumor cells with their microenvironment and induction of activated stroma are essential features of tumor progression. The mechanisms of these interactions have been studied in heterotransplants and organotypic cocultures of ras oncogene-transfected HaCaT keratinocytes. Consecutive in vivo passages as s.c. tumors provoked stepwise progression of benign to metastatic tumor cells associated with neo-expression of G-CSF and GM-CSF by tumor cells (1, 2). Both factors stimulated tumor cell proliferation and migration and enhanced stromal activation and angiogenesis. Comparably, overexpression of PDGF in non-tumorigenic HaCaT cells resulted in their progression to benign tumorigenicity via paracrine effects of the activated stroma concomitant with transient induction of angiogenesis (3). Ongoing angiogenesis and stroma activation with tumor vascularization are characteristic for malignant heterotransplants and a prerequisite for tumor cell invasion. Blockade of angiogenesis and tumor vascularization by different mechanisms arrested tumor invasion and expansion (4-6). This was associated with vessel maturation, fibrotic tissue formation and reconstitution of epithelial basement membrane. One consequence of stromal cell activation and leucocyte infiltration is enhanced extracellular matrix turnover caused by matrix-degrading proteases (MMPs), key players in the invasive process. The expression of different MMPs in benign and malignant cell clones in monocultures in vitro did not reflect their divergent growth behaviour in vivo. Malignant cells only enhanced MMP expression in fibroblasts both in cocultures in vitro and surface transplants in vivo (7, 8), whereas MMP expression in benign tumor cells was abrogated in vivo. Expression of different MMPs was consecutively induced by carcinoma cells in the stroma, indicating distinct functions in the invasion process. MMP expression occurred in perivascular cells and was predominantly localized in infiltrating white blood cells. Collectively, these data demonstrate a decisive role of the microenvironment for tumor progression and differential effects of benign and malignant cells on stroma activation and its cellular and matrix composition.

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The role of TGF-β in radiation responses

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Transforming growth factor-β1 (TGF-β1) is rapidly activated extracellularly following ionizing radiation exposure *in vivo*. We have identified a novel redox mechanism in which the latent protein undergoes a highly specific oxidation that releases TGF-β from the latent complex to bind to cell surface receptors that phosphorylate the R-Smad signaling pathway. Activation of TGF-β1 in irradiated tissue contributes to extracellular matrix remodeling and altered phenotypes that can contribute to either carcinogenesis or fibrosis. We used *Tgfβ1* knockout mice, exogenous inhibitors and specific immunostaining for latent and active forms to study specific effects of TGF-β in irradiated tissues. Our recent studies show that TGF-β1 plays a primary role in the cellular response to DNA damage. Radiation-induced apoptosis and cell cycle delay fail in epithelial tissues of *Tgfβ1*null mice. Consistent with the lack of appropriate cell fate decisions, p53 phosphorylations that are necessary for its stability and transcriptional activity are significantly reduced in tissues and cultured cells from *Tgfβ1* heterozygous mice. These data suggest that TGF-β1 should be considered a key regulator of genomic integrity since its loss during early carcinogenesis could contribute to genome instability through reduced action of p53. Furthermore, loss of TGF-β1 sensitivity in tumor cells could compromise their response to radiation therapy.

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Hypoxic tumour environment and its implication for cancer treatment

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The increasing availability over the past ten years of instruments and techniques to measure the oxygenation of human tumors has confirmed the prediction made 50 years ago by Thomlinson and Gray that human tumors would contain hypoxic regions. There is now no question that the majority of human tumors contain a significant fraction of viable cells that are at oxygen levels much lower than those of any normal tissues. This has profound implications for cancer therapy. First, the oxygen level of these tumor cells is well below the level that confers maximum radiosensitivity, and it has been shown by many investigators that the outcome of radiotherapy is worse in the more hypoxic tumors. There is also considerable basic science and clinical evidence that hypoxia leads to both increased resistance to cancer chemotherapy, as well as to increased rates of metastases. The good new, however, is that this unique feature of the tumor microenvironment can be exploited for targeted cancer therapy. This lecture will discuss the latest data on the mechanism of action and clinical results of tirapazamine, the first drug to enter the clinic that is specifically designed to kill hypoxic cells. I will also discuss a novel approach, based on using anaerobic bacteria that colonize the necrotic areas of tumors, to target anticancer drugs specifically to tumors. In summary, although hypoxia presently has a negative impact on cancer therapy, it is likely that in the not too distant future it will be an advantage that can be exploited for targeted cancer therapy.