of clonal evolution tumour heterogeneity is caused by genetic instability and phenotypic drifting. Thus, tumours arise from a single "mutated" cell which upon subsequent additional alterations gives rise to more aggressive subpopulations within the original neoplastic clone. These cells may leave a large number of offspring by chance, or new mutations may provide a growth advantage over the other tumour cells. Waves of such clonal expansion and selection drive the process. Therefore, any cancer cell can potentially become invasive and cause metastasis. This stochastic model predicts that the evolution of cancer cells is influenced by intrinsic (e.g. signaling pathways) or extrinsic (e.g. microenvironment) factors. These influences are unpredictable or random and result in heterogeneity in the cell phenotype or in the tumour initiating capacity. A key tenet of this model is that all cells of the tumour are equally sensitive to such stochastic influences. Moreover, tumour initiating cells cannot be identified prospectively or enriched for by for sorting cells based on intrinsic characteristics.

Recently, our understanding of tumour heterogeneity has been expanded through "the hierarchy model" which predicts that cancers contain a minority population of tumour initiating cells or cancer stem cells (CSC) that resist treatment and give rise to the bulk of the more differentiated tumour cells. Thus, a tumour can be considered a hierarchy defined by a maturation process analogous to normal tissue homeostasis. Therefore heterogeneity arises as a consequence of the presence of biological distinct classes of cells with differing functional abilities and behavior within the hierarchy. As opposed to the stochastic model the hierarchy model predicts that tumour-initiating cells can be identified prospectively and purified from the bulk of non-tumourigenic population based on intrinsic characteristics. The fact that most epithelial cancers are composed of cells that retain at least some level of differentiation suggests that the cancer stem cell generates a linage restricted progeny with a finite life span which nevertheless constitute the majority of the tumour. It follows that the bulk of the tumour would die out without being replenished from the cancer stem cells. Other than that little is known about the function of differentiated cancer cells.

Evidence will be presented here for the existence of a stem cell hierarchy in the normal breast and in breast cancer.

631 The effect of TGF-beta on glioma initiating cells

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The oncogenic effect of the TGF-beta pathway has prompted the design of several compounds to be used as anti-TGF-beta therapies in cancer. However, it is crucial to understand the molecular pathways implicated in the malignant role of TGF-beta in oncogenesis in order to select the patient population that may benefit from an anti-TGF-beta therapy. We have focused our studies on the role of TGF-beta in glioma. We have demonstrated that high TGF-beta-Smad activity is present in aggressive, highly proliferative gliomas and confers poor prognosis in patients with glioma. Moreover, we have observed that TGF-beta induces the self-renewal capacity of glioma-initiating cells (GICs). GICs are considered to be responsible for glioma initiation, maintenance and recurrence, and hence are optimal therapeutic targets against this deadly disease. We have discerned the mechanisms and molecular determinants of the regulation of GICs by TGF-beta using a transcriptomic approach and analyzing human glioma biopsies, primary cultured patient-derived tumour cells, and patient-derived GICs.

632 Regulation of self-renewal in cancer stem cells

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Recent findings support the concept that cells with the properties of stem cells (SC) are integral to the development and perpetuation of several forms of human cancer, and that eradication of cancer stem cells (CSC) may be essential to achieve cancer cure. However, direct proof of these concepts is still lacking, mainly due the scarcity of appropriate model systems.

We have recently defined a number of CSC-specific biological properties and underlying molecular mechanisms. These findings were generated using mouse models of two types of human cancer: (i) leukaemia, obtained by transgenic expression of the PML-RAR or AML1-ETO leukemia-associated oncogene; and (ii) mammary tumour, obtained by transgenic expression of the ErbB2 oncogene, and represent the rationale for the research activities proposed in this grant application.

1. Extended self-renewal of leukemic SCs, due to p21 up-regulation (Viale et al., Nature 2009). The self-renewal potential of normal SCs (measured as the number of times a single SC replicates during a life span without losing its regenerative potential) is intrinsically limited. This limit becomes experimentally evident as exhaustion of their regenerative potential when SCs are synchronously induced to hyper-proliferate, such as in serial transplantation. Self-renewal of CSCs, instead, is virtually unlimited, as inferred by their ability to support continuous expansion of the cancer clone and to be propagated inexhaustibly during serial transplantation. We have demonstrated that up-regulation of the cell-cycle inhibitor p21 is indispensable for maintaining self-renewal of leukaemia SCs (LSCs). Expression of leukaemia-associated oncogenes in normal hematopoietic SCs (HSCs) induces DNA damage and activates a p21-dependent cellular response that, in turn, imposes cell-cycle restriction and triggers repair of the damaged DNA. This effect of p21 prevents the physiological exhaustion of HSC self-renewal, which occurs in time owing to accumulation of DNA damage, and confers an advantage to HSCs when they hyper-proliferate, as it occurs during stress or after full transformation (for example, in the LSCs), thus explaining the role of p21 in the maintenance of the self-renewal potential of LSCs. These findings imply that cell-cycle-restricted LSCs are critical for the initiation and/or maintenance of the leukaemic clone, suggesting that targeting this compartment might be critical to disease eradication, and suggest that inhibition of DNA repair might be synthetic lethal with oncogene expression. However, it is not clear: (i) whether targeting of p21 in growing leukemias can lead to tumour regression, e.g. whether p21 or the p21-pathways are molecular targets for therapeutic intervention; (ii) which are the molecular mechanisms underlying the effect of p21 on LSCs; (iii) whether p21-upregulation in CSCs is a general mechanism of transformation and is also critical in the more common epithelial tumours.

2. Increased frequency of symmetric self-renewing divisions in ErbB2-mammary CSCs, due to attenuated p53-signaling (Cicalese et al., Cell 2009). Normal SCs accomplish their functions of self-renewal and differentiation through a single mitotic division ('asymmetric division'), in which one progeny retains SC identity, while the other (progenitor) undergoes multiple rounds of divisions before entering a post-mitotic fully differentiated state. We found that self-renewing divisions of CSCs are more frequent than normal counterparts, unlimited and symmetric, thus contributing to increasing numbers of SCs in tumoural tissues. SCs with targeted mutation of the tumour suppressor p53 possess the same self-renewal properties of cancer SCs, and their number increases progressively in the p53-null pre-malignant mammary gland. We showed that p53 signaling is attenuated in ErbB2-driven tumours, and that pharmacological re-activation of p53 induced restoration of asymmetric divisions in cancer SCs and tumour growth reduction, without affecting rates of apoptosis or proliferation on additional cancer cells. These data demonstrate that p53 regulates polarity of cell division in mammary SCs and suggest that loss-of-p53 in epithelial cancers favors symmetric divisions of CSCs, contributing to tumour growth. Molecular mechanisms underlying the effects of p53 on mammary SC polarity remain unknown. 3. Biological heterogeneity of breast cancers correlates with their cancer stem cell content (Pece et al., Cell 2010). Emerging evidence suggests that the number of CSCs within a given tumour can significantly vary from tumour to tumour. This is true not only in human tumours when using xenotransplantation assays, as discussed above, but also in syngeneic mouse models. In this context, CSCs can denote a small subpopulation of tumour cells (0.0001−0.1%, as for ErbB2 mammary tumours o leukemias associated with loss of Pten) or rather constitute a substantial proportion of the tumour mass (>10% in Ras- or myc-induced lymphomas). It is not clear though if the variation in the relative frequencies of CSCs is due to tumour type, specific genetic aberrancies in a given tumour, stage of disease progression or the specific experimental system used. We have recently showed that the heterogeneous phenotypical and molecular traits of human breast cancers are a function of their CSC content. Using an expression signature derived from purified normal mammary SCs (MSCs), we analysed breast cancer expression data sets, and found that we can stratify breast cancers on the basis of their biological (poorly differentiated G3 vs well differentiated G1) and molecular (basal-type tumours from other molecular types of breast cancers: ErbB2-type, luminal-A or -B) characteristics. Xenotransplantation experiments and immunohistochemistry using markers from the SC-signature directly demonstrated that G3s are enriched in CSCs. Notably, genes annotated as putative p53 targets were significantly enriched ($p < 0.002$) in the MSC-signature, suggesting that p53 is one component of the genetic program that accounts for the diversity of abundance of CSCs in tumours.

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Symposium

Gene expression and regulation

633 The contribution of dysregulated ribosomal gene transcription to malignant transformation

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Background: c-MYC plays a prominent role in cancer. Intriguingly, many of the genes regulated by this oncoprotein are associated with ribosome biogenesis and we have previously demonstrated that Myc regulates a major rate limiting step in this pathway, transcription of the 45S rRNA genes by RNA Polymerase I