pathway include targeting PI3K itself, the downstream regulator Akt, although inhibiting this crucial signaling node might result in toxicity, and other downstream components such as mTOR, integrin-linked kinase (ILK), phosphoinositide-dependent kinase-1 (PDK-1), p70S6 kinase and Forkhead/FOXO1. As with other molecularly targeted agents including imatinib mesylate (Gleevec) and trastuzumab (Herceptin), the success of PI3K inhibitor drugs will likely depend on the selection of cancer patients likely to be responders and non-responders based on genomic aberrations. The co-development of molecular markers determine early responders allowing triage to effective will increase utility of the targeted agents.

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Choline kinase is a novel prognostic marker and a therapeutic target in human cancer

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Cancer cells have altered signalling pathways that are usually associated to the loss of their ability to differentiate, become blind to apoptosis-triggering signals, and are hyper-reactive or autonomous to proliferation signals. However, in order to proliferate, they still need to replicate DNA and some key cellular components such as membranes. Furthermore, cancer cells have altered key metabolic pathways that may become very fragile. By contrast, the plasticity of normal cells is based on solid grounds where metabolic pathways are quite robust and efficient. A proper understanding of the most critical signalling and metabolic pathways required to maintain cancer cells, will facilitate the design of very specific strategies that may specifically affect cancer cells with very little effect on normal cells. One of such approaches will be discussed on the light of the metabolic control on one of the potential Achilles heels of cancer cells: the pathways that control phospholipids metabolism. Regardless of the specific signals that promote cell cycle entrance and DNA replication, all cancer cells require the simultaneous increase in phospholipids synthesis. Furthermore, a link between this pathway and the generation of potent toxic metabolites exists. Thus, this metabolic pathway becomes an interesting mechanism to control cancer cells viability. As a model for this novel antitumoral approach, the design of specific inhibitors to choline kinase, one of the enzymes involved in phosphatidylcholine synthesis, will be reported. The antitumoral effect of such compounds is fully supported by the understanding of their mechanism of action. The enzymatic system that regulates phosphatidylcholine synthesis is more complex that anticipated. Recent progress in this field will be discussed.

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The TGF-beta pathway in cancer

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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. 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 oncogenic role of TGF-beta in glioma. In some glioma tumours, TGF-beta acts as an oncogenic factor. We have demonstrated that high TGF-beta-Smad activity is present in aggressive, highly proliferative gliomas and confers poor prognosis in patients with glioma and we have discerned the mechanisms and molecular determinants of the TGF-beta oncogenic response using a transcriptomic approach and analyzing human glioma biopsies, primary cultured patient-derived tumour cells, and patient-derived glioma stem cells. We have observed that TGF-beta exerts its proliferative function through the induction of PDGF-B. Moreover, we have found that human glioma stem cell self renewal is regulated by TGF-beta. Glioma stem cells are considered to be responsible for glioma initiation, maintenance and recurrence, and hence are optimal therapeutic targets against this deadly disease. We have identified the molecular mechanisms that regulate the self-renewal capacity of glioma stem cells through TGF-beta.

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SYMPOSIUM

Role of microRNAs in cancer

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Functional genetic approaches identify cancerous miRNAs

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microRNAs (miRNAs) are potent post-transcriptional regulators of protein coding genes. Patterns of mis-expression of miRNAs in cancer suggest key functions of miRNAs in tumorigenesis. However, current bioinformatics tools do not fully support the identification and characterization of the mode of action of such miRNAs. To perform genetic screens for novel functions of miRNAs we developed a library of vectors expressing the majority of cloned human miRNAs and created corresponding DNA barcode arrays. In a screen for miRNAs that cooperate with oncogenes in cellular transformation we identified miR-372 and miR-373, each permitting proliferation and tumorigenesis of primary human cells that harbor both oncogenic RAS and active wild type p53. We provide evidence that these miRNAs are potential novel oncogenes participating in the development of human testicular germ cell tumors by numbing the p53 pathway, thus allowing tumorigenic growth in the presence of wild type p53. Recently, we have used a novel functional genetic approach and identified miR-221 and miR-222 (miR-221&222) as potent regulators of p27Kip1, a cell cycle inhibitor and tumor suppressor. Interestingly, high miR-221&222 levels appear in signatures of poor prognosis cancers. Using miRNA-inhibitors we demonstrated that certain cancer cell lines require high activity of miR-221&222 for the maintenance of low p27Kip1 levels and continuous proliferation. Thus, high levels of miR-221&222 promote cancerous growth by inhibiting the expression of p27Kip1. Last, we performed experiments to uncover metastasis promoting miRNAs. We describe the role of miR-373 in cellular migration and metastasis of breast cancers. Thus, we find functional genetic experiments extremely useful in the identification and characterization of cancerous miRNAs.

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Small RNAs in animal development

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Piwi interacting RNAs (piRNAs) are a class of small RNAs that in many species is abundantly expressed in the germline. We and others have shown that their biogenesis differs significantly from mi- and siRNAs in at least two steps. First, piRNAs carry a chemical modification at their 3' most terminal nucleotide. This modification, an O-methyl on the 2'OH group, is deposited by a homologue of the plant Hen1 protein. Second, piRNAs are not made by Dicer. Instead, strong evidence has been obtained in Drosophila that piRNAs are made through Piwi protein mediated cleavages, where one type of Piwi protein generates the 5' ends of a new piRNA that will be loaded into a different Piwi paralogue. This model has been named the ping-pong model and piRNA sequences from fish and mice display signatures that are consistent with it. We have analyzed piRNAs binding to both Piwi proteins, both in testis and ovary. A clear ping-pong signature emerges, with Ziwi binding to antisense piRNAs and Zili to sense. In addition, we find both Piwi proteins to interact, and have identified a number of other Zili interactors. Furthermore, Piwi protein localization is very dynamic, ranging from diffuse cytoplasmic localization to granular staining along the cell and/or nuclear membrane, and complete intra-nuclear localization at specific stages of germ cell development. These findings indicate that Piwi proteins in zebrafish likely affect both cytoplasmic, such as transposon mRNAs, and nuclear targets, for example chromatin. Nuclear effects of Piwi proteins are further supported by an oocyte specific block in meiosis displayed by a zili hypomorphic allele. Finally, we will present data from C. elegans that hint at an age-dependent effect of RNAi on the fidelity of meiosis and chromosome segregation during the first cell divisions.