Preclinical Science

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Lipid rafts as novel targets for anti-cancer therapy

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Background: For long time the plasma membrane has been undervalued as a relevant target for radiation-induced cytotoxicity. It has become evident, however, that this subcellular compartment is a source of bioactive molecules that can be generated upon exposure to radiation and may act as (apoptotic) second messengers. In addition, liquid-ordered dynamic membrane micro-domains enriched in sphingolipids and cholesterol, also known as lipid rafts, have been implicated in the recruitment and internalization of receptors, signaling molecules and certain (anti-cancer) drugs. To engage these membrane functions, rafts need to cluster into larger platforms. This process can be facilitated by radiation-induced hydrolysis of sphingomyelin (SM) into ceramide.
Material and Methods: We have recently established a crucial role for lipid rafts in targeting apoptotic pathways by alkyl-phospholipids (APL). APL (Perifosine, Miltefosine, Edelfosine) comprise a group of synthetic anticancer agents that are used for various clinical indications. Perifosine, for example, has recently been evaluated in combination with radiotherapy in a clinical phase I study. Its role as radiosensitizer is currently tested in a multicenter randomized phase II study. APL induce apoptosis in tumor cells and strongly enhance the radiation response in vitro and in vivo. Unlike conventional chemotherapeutic drugs, APL act at the level of cell membranes where they accumulate in lipid rafts. Following internalization, APL interfere with de novo phosphatidylcholine (PC) biosynthesis, which is essential for membrane homeostasis and cell survival. Disruption of raft integrity by cholesterol extraction or SM degradation, inhibits APL uptake and apoptosis induction.
Results: We have generated an APL-resistant cell variant, S49AR, that is unable to internalize APL via lipid rafts and reveals no impaired PC metabolism after APL treatment. Furthermore, these cells were found to be cross-resistant to apoptosis induction by other stimuli, including DNA-damaging agents (ionizing radiation, etoposide) and death receptor stimulation (CD95/Fas). Intriguingly, S49AR cells had lost the ability to synthesise sphingomyelin as a result of an impaired expression of the enzyme sphingomyelin synthase 1 (SMS1). In these S49AR cells, survival signaling pathways (MAPK/ERK, Akt/PKB) are upregulated, whereas pro-apoptotic signaling (SAPK/JNK) is reduced. Knock-down of SMS1 in S49WT cells by siRNA recapitulates the SM deficiency, impaired APL uptake and apoptosis (cross-)resistance. Conversely, normalization of cellular SM levels restored raft function, APL uptake and apoptosis sensitivity, including to radiation.
Conclusion: Our data point to a causal relationship between sphingomyelin synthesis, lipid raft function and susceptibility to apoptosis induction to a variety of anti-cancer agents. These studies define lipid raft as novel targets for anti-cancer therapy and apoptosis induction.

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TROP2 is a novel, potent stimulator of tumour growth and of metastatic spreading of human cancer

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Background: Trop-2 is a transmembrane calcium signal transducer, that plays a role in cell-cell and cell-substrate adhesion.
Material and Methods: We investigated the expression pattern of Trop-2 by DNA array, SAGE, Northern and Western blotting, flow cytometry, confocal microscopy and IHC analysis in experimental systems and in man. We explored its functional role by overexpression or down-regulation and by directed mutagenesis in transformed and/or metastatic cells in vivo.
Results: DNA array, EST, SAGE, RT-PCR and Northern blot analysis of human tumors revealed expression of the TROP2 gene in the vast majority of human cancers. A corresponding overexpression of the Trop-2 protein was demonstrated by a large scale IHC analysis of human tumors (1755 cases). Trop-2 was demonstrated to potently stimulate the growth of tumor cells, and its down-regulation by siRNA inhibited it. Deletion of the cytoplasmic region of Trop-2 abolished its growth stimulatory capacity, as mutagenesis of the S303 PKC phosphorylation site did. Proteomic and phosphoproteomic analysis demonstrated a Trop-2-dependent activation of PKCα, FAK and Raf1, modulation of ERK, MEK and p38 MAPK, and upregulation of NF-κB. In vivo imaging demonstrated a dynamic colocalization of PKCα and Trop-2 in membrane ruffles and podosomes. Dominant negative PKCα and PKCα siRNAs selectively abolished the Trop-2-induced growth demonstrating that PKCα plays a key role in Trop-2 signaling. Strikingly, comparative global gene expression analysis revealed that TROP2 was the only gene up-regulated across different metastatic models, tumor types and animal species. IHC analysis revealed a dramatic up-regulation in metastases from colon, stomach, breast and ovary tumors in man. To assess if Trop-2 plays a causal role in metastatic spreading, the metastatic potential of TROP2-transfected KM12SM colon cancer cells, orthotopically injected in nude mice, was assessed. TROP2-overexpressing cells indeed demonstrated a profoundly increased metastatic potential to the liver. Deletion of the HIKE domain of Trop-2 severely diminished, whereas that of the whole cytoplasmic region vastly increased metastatic diffusion, indicating the existence of metastatic enhancers and silencers in the Trop-2 cytoplasmic tail.
Conclusions: Our findings demonstrate the existence of a previously unsuspected, strikingly widespread mechanism of stimulation of tumor growth and of metastatic spreading in man, and candidate Trop-2 for novel diagnostic and therapeutic procedures.

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PCAF plays a key role in the regulation of the cellular fate in hypoxia

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Background: The p53 tumour suppressor is one of the most extensively studied transcription factors involved in several crucial cellular functions such as cell cycle arrest, apoptosis, differentiation, or senescence. Although in hypoxic conditions p53 is stabilised the same way as under DNA damage it is incapable of inducing the expression of its pro-apoptotic target genes, including members of the Bcl-2 family. The aim of this study is to investigate the p53 transcriptional activity and specifically its inability to induce expression of several of its target genes including pro-apoptotic members of the Bcl-2 family under hypoxia.
Materials and Methods: In order to approach this question we used transcriptional regulation and gene expression methodology such as chromatin immunoprecipitation, luciferase reporter assays and cell cycle analysis.
Results: In this study we present evidence that p53 is hypo-acetylated at K320 site, which is targeted by PCAF, whereas efficiently acetylated at K382 by p300/CBP under hypoxia-mimicking conditions. Using several transcription assays we demonstrate that the acetylated p53 at K320 is not recruited to the BID promoter and hence p53 is incapable of activating this pro-apoptotic Bcl-2 family target. On the contrary, the limited amounts of acetylated p53 at K320 are still recruited to the promoter of the cell cycle arrest gene p21WAF and the expression of this cyclin/cdk inhibitor appears to be unaffected by hypoxic conditions.
Conclusions: Since the K320 p53 acetylation is the mainly affected site in hypoxia we conclude that PCAF HAT activity is the key regulator of the cellular fate under these conditions. Furthermore, the decision between apoptosis or cell cycle arrest is determined by the selective recruitment of a higher proportion of the K320-acetylated p53 to the p21WAF promoter in hypoxia. This provides an additional molecular mechanism explaining cell survival in hypoxic conditions.

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Intestinal inactivation of canonical Notch signaling by removal of protein O-fucosyltransferase 1 triggers secretory cell fate differentiation

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The intestinal epithelium is a continuously differentiating tissue in which the high rate of cell self-renewal contributes to the susceptibility of intestinal epithelial cells to malignant transformation. Therefore understanding mechanisms that regulate cell proliferation and maturation towards the different intestinal cell lineages is critical to understand intestinal tumorigenesis. Here, we report that active canonical Notch signaling in