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Radiotherapy and apoptosis

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The cellular response to radiation is complex and involves many signal transduction pathways. As a consequence of DNA damage, cell cycle arrest occurs, allowing time for DNA repair before mitosis takes place. If repair fails, several outcomes are possible: apoptosis, senescence, mitotic catastrophe and transformation. Which molecular factors determine this cellular decision between life and death, remain largely unknown. It has been suggested that the balance between survival and pro-apoptotic signals plays a critical role in dictating the cellular fate. As a result of our increased understanding of cell death and survival regulatory mechanisms, several strategies have been pursued to manipulate apoptosis and increase therapeutic outcome.

A number of novel signaling-based therapeutic agents have been developed and tested in combination with radiation. For example, *EGFR blocking agents* that inhibit mitogenic signaling and induce apoptosis are currently being evaluated as radio- or chemosensitizers in patients with EGFR overexpressing tumors. Another approach involves membrane-targeted synthetic antitumor lipids, like *Perifosine*. These agents increase apoptosis sensitivity and cause tumor regression when given concurrently with radiation. Clinical phase II testing of this combination therapy is underway. The *death receptor ligand TRAIL* is also of extreme interest due to its proven capacity to induce apoptosis in a variety of tumors, but its lack of normal tissue toxicity in preclinical models. TRAIL is an excellent candidate for combination therapy, since TRAIL and radiation activate partially distinct death pathways, while a molecular basis for synergy lies in p53-dependent and -independent upregulation of the TRAIL-R by radiation. This concept is currently being studied in several *in vitro* and *in vivo* models. To visualize and monitor tumor response induced by these various apoptosis-modulating agents, we have evaluated a novel non-invasive *in vivo* imaging technique: *99mTc-Annexin V (TAV) scintigraphy*. In a series of 65 patients with various types of cancer, we established a significant correlation between tumor TAV uptake and treatment outcome, suggesting a predictive value of this test.

Conclusion: Our improved understanding of the mechanisms involved in apoptosis has allowed the rational design of novel therapeutic strategies. This provides an exciting opportunity to introduce a new generation of (radio-)biological response modifiers in clinical studies.

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TRAIL area

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Efficacy of chemotherapeutic drugs is hampered by occurrence of drug resistance. Several mechanisms cause drug resistance. A final common factor is the reduced capacity of resistant cells to go into apoptosis following treatment with DNA damaging agents. This common factor makes it of interest to search for ways that facilitate the cell to go into apoptosis following chemotherapeutic drugs. Apoptosis is induced by multiple stimuli including growth factor withdrawal, irradiation, chemotherapeutic agents, or activation of death receptors. Apoptosis is executed by activation of effector proteases, caspases, which cleave specific death substrates resulting in cellular disassembly. Apoptosis can be executed through a mitochondria-dependent (instrinsic) and a mitochondria independent (extrinsic) pathway. The "extrinsic" pathway is initiated by activation of death receptors on the cell membrane. The death receptor ligands TNF, FasL and TRAIL can induce apoptosis by binding to their cell membrane receptors. Recombinant forms of these ligands potentiate chemotherapeutic drug effects in preclinical models. For the application of recombinant human (rh)TNF, FasL and TRAIL in patients, it is of primary importance that their safety in the clinical situation is guaranteed. High dose rhTNF has shown low antitumor activity and severe sepsis-like toxicity. But rhTNF plus melphalan is used for local tumor treatment by limb perfusion. Because of severe liver toxicity in mice due to Fas-mediated apoptosis of hepatocytes rhFasL is not tested in humans. TRAIL currently produced as soluble, zinc stabilized rhTRAIL without His-tag seems to be without preclinical toxicity. TRAIL can be present in serum of SLE patients and after endotoxin challenge. This illustrates that the human body can tolerate certain TRAIL levels. A phase I study with rhTRAIL is initiated. Currently agonistic DR4 and DR5 antibodies against the DR4 and DR5 TRAIL death receptors are studied in the clinic as another option to induce apoptosis. The two ongoing phase 1 studies with the agonistic DR4 antibody showed that it is well tolerated and the MTD is not yet reached. With the agonistic DR4 antibody, apart from ongoing phase 1 studies there are phase 2 studies in NSCLC, colorectal cancer and Non-Hodgkin's lymphoma. In the ongoing non-Hodgkin's lymphoma study 3 partial tumor responses have been observed. In addition there are 2 ongoing phase 1b studies in combination with chemotherapy. Because of the synergistic effect observed in the preclinical setting between death receptor ligands and chemotherapy, it may well be that death receptor ligands are especially active and of value in the clinic if combined with chemotherapy. Hopefully choices for specific (modified) death receptor ligands for the treatment of patients can in the future be rationally made based on tumor characteristics.

Scientific Symposium
Malignant glioma – from bench to bedside and back

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New insights into DNA and chromosomal changes in brain tumours

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Studies of DNA and chromosomal changes in brain tumors have been limited by the material available and the work involved. Array technology permits the assessment of copy number status across the genome at a resolution previously impossible despite limited material. Combining this technology with microsatellite/SNP analysis (allele information) and FISH (information on structural rearrangesments consequent on losses and gains/amplification) provides a method for understanding the changes associated with cancer development. Recently we examined more than 100 adult diffuse astrocytic tumors using a 1Mb whole genome array and confirmed a high frequency of chromosome 6 and 22 copy number abnormalities. To map the abnormal region(s) that potentially harbor tumor suppressor gene(s) or oncogene(s), we constructed tile path arrays covering 98.3% of chromosome 6 sequences and 83.8% of 22q. Data from these arrays and microsatellite analysis showed the alterations on both chromosomes are complex: combinations of deletions with or without reduplication of a retained allele, as well as copy number gains and amplifications. Two novel overlapping homozygous deletions on 22q were identified that involved three genes (DEPDC5, YWHAH, C22ORF24). Chromosome 6 abnormalities were predominantly deletions of the q arm and two small common and overlapping regions of deletion at 6q26 were identified. One 1002 kb in size contains PACRG and QKI, while the second was smaller containing a single gene, ARID1B. The advantages of combining array-CGH and microsatellite/SNP analysis in elucidating complex genomic rearrangements in tumor tissue is clearly demonstrated and ongoing FISH studies are analyzing the structural rearrangements consequent on these losses and gains. Another area of great interest is methylation of gene promoters resulting in decreased expression. The DNA repair enzyme O6-methylguanine DNA methyltransferase (MGMT) is an example, as this enzyme may cause resistance to DNA-alkylating drugs used in the treatment of gliomas. Combining methylation analysis with real-time reverse transcription-PCR and immunohistochemical or western blot analysis demonstrates the presence and consequences of promotor methylation on protein expression. Application of these technologies to brain tumors will further our understanding of their biology and provide prognostic indicators, indicators of response to therapy as well as identifying new thereputic targets.

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Glial stem cells and malignancy

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A highly infiltrative cancer stem cell phenotype was established by xenotransplantation of human brain tumors in immuno-deficient nude rats. These tumors coopted the host vasculature and presented as an aggressive disease without signs of angiogenesis. The malignant cells expressed neural stem cell markers and showed a migratory behavior similar to normal human neural stem cells. The cells showed self-renewal capacity and gave rise to tumors in vivo. Serial animal passages, gradually transformed the stem cell tumors into an angiogenesis-dependent phenotype. This process was characterized by a reduction in stem cells markers. Pro-invasive genes were up-regulated and angiogenesis signaling genes were down-regulated in the stem cell tumors. In contrast, pro-invasive genes were down-regulated in the angiogenesis-dependent tumors, derived from the stem cell tumors.