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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

86 INVITED

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.

The described angiogenesis-independent tumor growth and the uncoupling of invasion and angiogenesis, represented by the cancer stem cells and the cells derived from them respectively, points at two completely independent mechanisms that drive tumor progression. The present work underlines the need for developing therapies that specifically target the cancer stem cell pools in tumors.

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Exploiting biological targets for therapy

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Current treatment of glioblastomas relies on surgical resection, radiotherapy and chemotherapy. However, the efficacy of these treatments is still limited and new therapeutic approaches based on the understanding of brain tumor biology are emerging. High expression of the EGF receptor by tumor cells, activation of the PI3K/Akt and the Ras/Raf pathways, secretion of protease by the tumors represent interesting targets for new selective drugs under development. Numerous antiangiogenesis agents are currently in preclinical development and early clinical trials, including VEGF and VEGFR antibodies and small molecule inhibitors.
So far, most of these new drugs have shown disappointing results in phase 2 trials. Combination of these drugs, or association with radiotherapy or chemotherapy might increase their efficacy. Translational research will probably identify sub-groups of patients with specific tumor molecular profiles, allowing tailored or specific therapies based on collections of genetic alterations. In addition, the recent development of convection-enhanced delivery technique allows the administration of drugs which do not cross the blood-brain-barrier, such as selective toxins, antisens or immunostimulating oligonucleotides. Some of these drugs are currently being tested in randomized phase 3 trials.

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Exploiting biological targets together with radiation

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Glioblastoma multiforme (GBM) and high grade astrocytomas are heterogeneous tumors and are characterized by areas of severe hypoxia by virtue of abnormal tumor vasculature giving rise to poor tumor blood perfusion and high interstitial fluid pressure. This represents significant challenges for cytotoxic therapy. It is well appreciated that hypoxia leads to radioresistance because of lack of oxygen to facilitate DNA damage by radiation-induced free radicals. In addition, hypoxic conditions create a microenvironment in which tumor cells become more angiogenesis dependent, more apoptosis resistant, more capable of existing under hypoxic conditions and more malignant because of the development of genomic instability and mutant genotypes impacting on apoptosis/survival signaling pathways. In GBM, the mutant genotype includes loss of the PTEN tumor suppressor, constitutive activation of the PI3K/Akt/mTOR signaling pathway and EGFR upregulation, amplification and/or mutation. These genetic changes can influence the response to ionizing radiation. Preclinical data will be presented on the use of signal transduction molecules including antiangiogenic therapies, combined with ionizing radiation. Issues including the spectrum of preclinical models, optimal timing of targeted agents and the integration with chemotherapy, will be discussed. Finally, novel phase I strategies to move laboratory findings rapidly into the clinic will be presented.

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Biological predictors for chemotherapy response

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Clinical parameters (age, performance status, tumor resectability, histology + tumor grade) are important prognostic factors. Understanding of tumor biology and identification of molecular markers are crucial for future trials. However, many markers may merely reflect a different natural history and do not contribute to individual patient management. Oligodendroglioma with LOH 1p/19q has been associated with high response rates and improved survival, although the underlying genetic defect has not been identified yet. In anaplastic oligoastrocytoma and oligodendroglioma 2 trials evaluated PCV-chemotherapy (before/after radiotherapy (RT)), compared to initial treatment with RT alone. Neither trial demonstrated improved survival with early chemotherapy administration; progression-free survival was prolonged in the chemotherapy groups. Even in the subset of patients with LOH 1p/19q, considered the most sensitive to chemotherapy, no improvement was seen. These patients had prolonged overall survival irrespective of treatment, thus characterizing a distinct pathologic entity. Improved outcome has also been suggested for patients with a methylated promoter of the DNA repair gene MGMT. Tumors with a methylated MGMT gene promoter, thus a silenced gene are unable to repair some of the DNA damage induced by the chemotherapy. In glioblastoma improved survival was demonstrated for temozolomide (TMZ) and RT. We showed that the benefit of the addition of TMZ chemotherapy was almost exclusively confined to patients whose tumors had a methylated (silenced) MGMT promoter. At 2 years, 46% of the pts treated with TMZ/RT and whose tumors were MGMT methylated survived, compared to only 14% for the pts with unmethylated tumors. Small molecule drugs targeting signaling pathways aberrantly activated in tumors will be central to future clinical trials. In glioblastoma the EGFR- and PI3K-pathway represent attractive targets. Promising are also strategies that aim at angiogenesis such as inhibitors of VEGF(R) or of integrins. It is likely that combination treatments are required. Since these strategies aim (mainly) at specific molecular targets it is mandatory to establish molecular profiles of the tumors for individualized treatment. Thus, common to all ongoing or planned trials is the absolute necessity of availability of tumor material for molecular profiling (paraffin-embedded or ideally fresh-frozen) in order to provide the patient the most adapted treatment.

Scientific Symposium
Pelvic relapses in gynaecology

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Overview of pelvic relapses in gynaecological cancer

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Presentation of pelvic relapses from gynecological malignancies depends on the origin of the primary tumor.
Ovarian cancer rarely relapses in the pelvis alone but the pelvic relapse may represent significant tumor burden. These recurrences are largely intraperitoneal. Nodal disease on the sidewalls as a sole site of recurrent tumor is rare in ovarian cancer. Management is going to depend on the treatment free interval since primary therapy. Surgery is an option with treatment free intervals greater or equal than one year, dependent on patient operability and the likelihood of being able to perform a complete tumor debulking. Radiation therapy is used in the exceptional case where relapse occurs in the pelvis only. Most patients will be managed by systemic chemotherapy.
In the tumors of vulva, endometrium and cervix, isolated pelvic relapse may occur with cervix cancer contributing to the majority of such patients. While radiotherapy is most frequently the initial treatment option chosen, it is important to distinguish two different forms of recurrence: A. Central occurrence, B. Sidewall occurrence.
Pelvic sidewall recurrence represents a challenging problem. Often characterized by invasion of adjacent muscle, vessels nerves and other organs, a variety of surgical options have been explored in these circumstances. While local control appears to be feasible more frequently with extensive surgery, extensive sidewall disease is also frequently characterized by occult hematogenous and/or lymphatic metastasis extending beyond the resectable area. New diagnostic techniques, for example, PET scanning may help to better select patients for this type of radical procedure. For most of the patients with recurrent tumor involving the pelvic sidewall, the available approach remains strictly palliative after the failure of radiation therapy. This would be the first treatment option as it still may offer a chance of cure in the not previously radiated patient. Chemotherapy and/or other medical interventions are strictly palliative in nature, while rare long term survivors have been reported with prolonged chemo- or hormonal therapy. Central recurrence, also after radiation therapy may be amenable to supra-levatorial or trans-levatorial exenteration giving the patient with this type of recurrence a 50% chance of cure if all tumor can be surgically resected. Expand the indications for radical surgery to encompass also some extension towards the sidewall. Also, the array of available reconstructive options has largely increased in recent years culminating in the concept “stoma-less exenteration”. This has greatly enhanced the acceptability of such procedures for the patient and contributed to a better quality of life.
In summary, pelvic relapse of gynecological tumors represents a challenging clinical situation that mandates individualized treatment options. Therefore, therapy should be performed at large referral centers.