4 INVITED

Pelvic MRI: how MRI has helped develop new treatments

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The introduction of new radiotherapy and surgical techniques requires close collaboration between clinician and radiologist to optimise the application of imaging. This principle will be illustrated by a review of how MRI has helped implement new therapies for the treatment of cervical and rectal carcinoma. In cervical cancer the selection of patients for uterus-conserving surgery has been optimised by accurate MRI prediction of the relationship of the tumour to the internal os. Also, intensity-modulated radiotherapy (IMRT) has been aided by co-locational imaging between MRI and CT to define the gross tumour volume (GTV). Widespread implementation of pelvic IMRT has been prevented by the lack of a validated method for defining the nodal clinical volume (CTV). Recent work has shown the accuracy of ultrasmall paramagnetic iron oxide particles (USPIOs) in distinguishing infiltrated from normal nodes in gynaecological malignancy. By evaluating 1216 nodal contours, using USPIOs, guidelines for outlining pelvic nodes have been produced.
Multi-institutional studies in rectal cancer show that MRI is equivalent to histopathology in delineating local tumour spread prior to total meso-rectal excision. As the presence of the tumour at the circumferential resection margin affects prognosis, MRI plays a crucial role in the selection of appropriate therapy and has become an effective tool for the identification of patients at risk of incomplete resection.

5 Varian Award

Clinical Varian Research Award Lecture – Repair of DNA double-strand breaks in radiation induced cellular damage

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DNA damage, especially double-strand breaks, can be induced by endogenous or exogenous damaging agents, such as ionizing radiation. Repair of DNA damage is very important in maintaining genomic stability. Incorrect repair may lead to chromosomal aberrations, translocations and deletions. Consequently, incorrect repair might result in oncogenic transformation of cells, which can lead to the development of cancer. Thus, unreaveling the pathways of double-strand break repair is essential in understanding the genetic interactions that lead to oncogenic changes. Biochemical studies have provided insight into the molecular mechanisms by which various proteins, involved in repair of double-strand breaks, perform these essential tasks. The next step ahead is analyzing the relationship between the individual biochemical activities of double-strand break repair proteins and their coordinated action in the context of the living cell. This presentation describes the cellular behaviour and cooperation of the mammalian double-strand break repair proteins Rad51, Rad52, Rad54 and Mre11 after induction of DNA damage by ionizing radiation. Furthermore, the possibility to use detection of these proteins by ionizing radiation-induced foci formation and determination of telomere length as methods that might possibly serve as a predictive assay for measuring individual radiosensitivity in humans, was examined.

6 Varian Award

Clinical Varian Research Award Lecture – Molecular markers of tumor hypoxia: predictors of clinical radiation resistance?

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In the last five years, several groups investigated the role of proteins involved in the "hypoxic response" of tumor cells as molecular markers of tumor hypoxia. The hypoxia-responsive transcription-factor subunit hypoxia-inducible factor-1α (HIF-1α) itself and the products of HIF-1-regulated genes, such as carbonic anhydrase IX (CA IX) and glucose transporter 1 (GLUT1) were most frequently studied as endogenous hypoxia markers by immunohistochemistry, mostly in archival material. Although results were not uniform, the majority of series suggested an association of high marker expression with poor outcome (e. g. overall survival, disease-free survival, rarely local control) or with low tumor oxygenation measured with oxygen electrodes.
In detailed *in-vitro* studies of the accumulation of HIF-1α and CA IX protein in human tumor cell lines, we showed that these markers respond already to very mild hypoxia (e. g. 5% O_2), but have distinct time courses of accumulation and degradation as well as different patterns of response to further decreasing O_2 concentrations. Therefore, an association of marker expression and hypoxic radiation resistance *in vitro* appeared to be cell-type specific. As varying degrees of colocalization of these molecular markers with injected hypoxia markers (pimonidazole, EF5) were observed in tumors, we investigated the effect of manipulating non-hypoxic tumor microenvironment conditions (pH, glucose and serum availability) on the hypoxic HIF-1α and CAIX accumulation *in vitro*. Our observation of a strong glucose dependency corresponds to an immunohistochemical pattern of a lack of endogenous marker expression in hypoxic areas most distant from blood vessels observed by some groups. Despite these limitations, the activation of hypoxia-responsive elements (HRE) by HIF-1 has been successfully used in hypoxia reporter assays and to specifically target hypoxic tumor cells by gene therapy. Targeting HIF-1α pharmacologically is an emerging therapeutic strategy, the success of which will depend on the hypoxic specificity of HIF-1α accumulation.
More recently, osteopontin has been suggested as a plasma marker of tumor hypoxia with a surprisingly strong association with both tumor oxygenation and outcome. Although a ras-activated enhancer has recently been shown to mediate the hypoxia-enhanced transcription of osteopontin, we observed no increase of osteopontin in the culture medium during severe *in-vitro* hypoxia in a panel of human tumor cell lines.
Ideally, hypoxia markers should identify those tumors with the lowest oxygenation and such patients should be treated with effective therapies targeted at hypoxic tumor cells. First reports suggest that this combination may work for pimonidazole and ARCON (accelerated radiotherapy, carbogen, nicotinamide) as well as for osteopontin and the radiosensitizer nimorazole, whereas data on HIF-1-related markers in the context of hypoxia-specific treatment are less promising so far.

ESSO Special session

7 ESSO Award

Rectal cancer treatment in 2005

L. Påhlman. *University Hospital Uppsala, Department of Surgery, Uppsala, Sweden*

Rectal cancer treatment has changed dramatically during the last twenty years. The introduction of more precise surgery (TME technique) as well as a more sophisticated use of radiotherapy has encountered this change.
Staging: Before any patient is treated for rectal cancer it is important to have a proper staging process. First of all the liver and lung have to be scanned to disclose any distant metastases. Secondly, a preoperative MRI will guide the use of radiotherapy and what type of surgical technique. In very tiny lesions (T1-lesions) endorectal ultrasound will disclose whether this is possible or not to do a local excision.
Surgery: The tradition of blunt dissection technique has now been abandoned, mainly due to the risk of high local recurrence rate. It has been proven that the rectal fascia is a barrier for tumour spread and provided that the whole mesorectum and rectum are taken out without any tears of the rectal fascia the local recurrence rate would be reduced dramatically. This technique (TME technique) has been introduced during the last 20 years and the main thing is to follow the avascular anatomical planes and divide the inferior mesentric artery close to the aorta. In tumours situated in the upper third of rectum a TME is probably not necessary and in those cases a resection with 5cm distal margin in the mesorectum is essential. In very low rectal cancers, the 5cm rule is not necessary, since there is no mesorectum harvesting lymph nodes and there is no evidence that a rectal adenocarcinoma will grow more than 5 mm from the macroscopic margin. Consequently, more sphincter preserved procedures can be done if this rule is accepted.
Radiotherapy: Overwhelming data have shown that preoperative radiotherapy is more dose-effective in terms of reduce of the local recurrence rate and also at the end beneficial for survival. Two main options occur. One is the short course with one week treatment and the other is a long course. There are no data indicating that either of them should be the better. An important topic is whether additional chemotherapy to radiotherapy would improve the effect. There are data indicating that the local recurrence rate will be reduced but this has no real impact on survival. On the other hand, it is known that toxicity will increase substantially if chemotherapy is added to radiotherapy.
Another important topic is whether preoperative radiotherapy of preferably chemoradiotherapy will increase the number of preserved sphincters. Uncontrolled data support that but all randomised trials specifically addressing this question have not shown a beneficial effect with chemoradiotherapy compared to radiotherapy.
Future: Based upon available literature the problem with the high local recurrence rates in rectal cancer surgery has been more or less solved. With appropriate surgical technique and selective use of radiotherapy the local recurrence rate should not exceed 5%. However, despite this the mortality due to distant metastases (30% of those operated upon

radically) has been constant despite to this change in surgery and the use of radiotherapy. Therefore, the next step will be to take into account the risk of distant metastases. Based upon preoperative staging upfront chemotherapy might be a solution.

Pezcoller Foundation/FECS recognition for Contribution to Oncology

8 Pezcoller/FECS Award

Functional genetic approaches to cancer

R. Bernards. *Division of Molecular Carcinogenesis. The Netherlands Cancer Institute, Amsterdam, The Netherlands*

The quantum leap forward in our understanding of the molecular basis of cancer over the past two decades has not yet been accompanied in a comparable increase in our ability to diagnose or treat cancer. In this lecture I will illustrate how we can exploit the power of genetics and genomics to greatly improve the diagnosis of cancer and develop new and far more powerful classes of anti cancer drugs.

Better diagnostics: Cancer is a disease that results from changes in cellular gene expression. As the behavior of cancer cells is determined by the expression of their genome, the pattern of gene expression may reveal many traits of individual cancers, including responses to anti-cancer therapies and propensity to form distant metastases. In the past, numerous clinical studies have correlated expression levels of individual genes with disease outcome. In general, the results of these studies have been disappointing. This indicates that individual genes have only limited predictive power and points to the need for a multi-gene-based approach. We have used DNA micro-array technology to obtain detailed insights into the behavior of tumors. By analyzing patterns of gene expression in a series of breast cancer of varying aggressiveness, we were able to identify a 70-gene signature that predicts the development of distant metastases in breast cancer. This gene signature has been developed into the first micro-array based diagnostic test for cancer. The availability of clinically-useful and validated gene signatures will help breast cancer patients in making difficult therapy choices.

Our current efforts in this area are focused on the identification of gene expression profiles that predict responses to new generations of anti-cancer drugs, the targeted therapeutics. These drugs often target specific molecules that are hyper-active in cancer (examples are the HER2/NEU receptor in breast cancer, the BCR-ABL kinase in chronic myeloid leukemia and the EGF receptor in lung and colorectal cancer). These drugs are often very effective and have very few unpleasant side effects other than their steep price. It is widely believed that in the next 5 to 10 years the clinical application of these new targeted therapeutic agents will personalize, and thereby revolutionize, the care for cancer patients The price issue is a serious one, as it becomes increasingly clear that there is a limit on the amount of money society can spend on cancer drugs. The development of diagnostics to identify those patients that benefit most from expensive targeted therapeutics may help solve this societal dilemma.

Better therapeutics: One of the major remaining deficits in our understanding of the human genome is that information regarding gene function is available for only one quarter of the approximately 30,000 genes. Many of these hitherto anonymous genes are potential targets for the development of new anti-cancer drugs. My laboratory has developed functional genetic approaches to obtain information regarding gene function using high-throughput screens. We have developed both gain-of-function genetic screens and loss-of-function genetic screens to carry out large-scale genetic screens in mammalian cells. We focus on the central growth-regulatory pathways that are most frequently deregulated in cancer.

One attractive new opportunity provided by the new genetic tools available to cancer geneticists is that it allows us to identify completely new and innovative classes of anti cancer drugs. One concept that was formulated as early as 1997 by Hartwell and Friend is that of genotype-specific drug targets, *i.e.* targets whose inhibition is only toxic to cells carrying a defined (cancer-specific) genetic lesion. In theory, such drugs should be far more selective for cancer cells than the current generation of broadly-acting cytotoxic drugs. Unfortunately, this concept of "synthetic lethal" interactions has remained a subject about which more reviews have been written than solid data published. Nevertheless, given the frequent occurrence of synthetic lethal interactions in yeast, such interactions will sooner of later also be found in mammalian cells. I will discuss our own efforts in identification of new classes of drug targets using large-scale RNA interference screens in mammalian cells.

Young Oncologists session
How to select a target for chemoprevention? Illustration based on mTOR pathway inhibition

9 INVITED

mTOR pathway and cancer: general concepts

M. Pende. *Inserm Avenir, U584, Faculté de Médecine Necker, Paris*

Target of Rapamycin (TOR) is a serine/threonine kinase whose function is conserved from yeast to metazoans. Depending on nutrient availability, TOR regulates cell number and size, by promoting cell cycle progression, cell survival and anabolic pathways. In mammals, the insulin and insulin-like growth factors (IGFs) also participates in TOR regulation via the Insulin Receptor Substrates (IRS), phosphatidyl inositide 3 kinase (PI3K) and the small GTPase Rheb. Mammalian TOR (mTOR) exists in two complexes. The first includes the raptor protein, is inhibited by the macrolide antibiotic rapamycin, and phosphorylates the S6 kinase (S6K) and eIF4E-binding protein (4EBP) families. The second complex includes the rictor protein, is insensitive to rapamycin and phosphorylates the Akt (PKB) kinases, as well as other kinases of the AGC family. By using mouse genetics and rapamycin, our group is addressing how pathophysiological growth and proliferation are controlled by this pathway. We show that muscle cell size and cell number are regulated by separate branches of the mTOR pathway and that S6K1 is selectively required for size control. Since tumours having deregulated activity of the PI3K/Akt pathway are often extremely sensitive to rapamycin, our group is also addressing the role of S6K1 in tumorigenesis. We have characterised a mouse model of insulinoma by overexpressing the oncogenic form of Akt under the rat insulin promoter (RIP-MyrAkt1). These mice develop tumors, starting at four months of age and progressing to 60% of incidence and decreased viability after ten months. Strikingly, the S6K1 deletion is sufficient to block pancreatic beta cell tumorigenesis. In conclusion, we propose S6K1 may serve as a mTOR effector promoting growth and tumorigenesis.

10 INVITED

Rapamycin analogs in cancer therapy

J. Tabernero, J. Baselga. *Vall d'Hebron University Hospital, Medical Oncology Department, Barcelona, Spain*

mTOR has been shown to be a key kinase acting downstream of the activation of the phosphatidylinositol 3 kinase (PI3K). In humans, mTOR is a nutrient sensing protein acting as a master switch of cellular catabolism and anabolism, signaling cells to multiple critical effects including cell growth, proliferation and survival. Rapamycin and its analogs (CCI-779, RAD001 or everolimus, and AP23576) are macrolides that block mTOR. Initially, these compounds were developed as immunosuppressive drugs. Interestingly, rapamycin and its derivatives have been shown to inhibit the growth of several human cancer cell lines in preclinical models. Based on this preclinical activity, rapamycin and its analogs are being clinically developed as anticancer drugs. These compounds have been evaluated in both continuous and intermittent schedules. They have a favorable safety profile with skin rash as the most frequent side-effect, the dose-limiting toxicities being mainly thrombocytopenia, mucositis, and asthenia. Evidence of long-lasting antitumour activity has been reported in patients with breast cancer, renal cell carcinoma and colon cancer. This clinical activity has not been shown to be dose- or schedule-dependant. Surrogate downstream and upstream molecular markers are being used to monitor the biological effects of rapamycin derivatives in order to determine the optimal biological dose with these compounds, although that preliminary evidence suggest that this approach might be insufficient to predict response. The molecular characterization of the activation status of m-TOR related signaling pathways as well as the mutational status of some selected genes might provide critical information to identify the population that might be more sensitive to these compounds. Rapamycin analogs are also being developed in combination with hormone agents, chemotherapy and other targeted agents. Some preclinical studies have elegantly defined opportunities for these combinations with the simultaneous inhibition of multiple signaling pathways thereby preventing resistance induced by intracellular crosstalk and signaling redundancies. In summary, m-TOR inhibitors are agents with anti-cancer activity and a favorable safety profile. Through a better understanding of the m-TOR-related signaling pathways and the precise knowledge of the potential synergistic or additive interactions with other drugs, the clinical development of rapamycin analogs will continue to expand.