29 INVITED

Cervix cancer screening in low-resource settings

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Countries in South and Central America, Sub-Saharan Africa and South and South-East Asia account for more than 80% of the world-wide burden of cervical cancer. The risk of disease and death from cervical cancer has remained largely uncontrolled in high-risk developing countries due to lack of or inefficient screening programmes. Consequently, precancerous lesions are rarely diagnosed and treated and invasive cancers generally present at advanced stages of disease with poor survival. Cytology screening programmes in developed countries have resulted in dramatic reduction in the burden of cervical cancer. However, cytology testing requires complex inputs in sample collection, processing, reading and reporting of smears. Cytology-based screening programs have been introduced in some developing countries, particularly in South and Central America, over the last three decades. Generally, they have achieved very limited success in preventing incidence of and mortality from cervical cancer in those regions. The findings from studies addressing the comparative performance of conventional cytology and its potential alternatives such as visual inspection with acetic acid (VIA), magnified VIA (VIAM), visual inspection with Lugol's iodine (VILI) and HPV DNA testing in detecting cervical cancer and its precursors will be discussed in the context of evolving public health policy on introducing new and effective programs in low-resource settings and in re-organizing existing programmes. The accuracy of VIA and VILI seem to be similar to that of good quality cytology in most recent studies in developing countries Early findings from randomized trials evaluating these tests for their effectiveness in reducing incidence of and mortality from cervical cancer will also be described. Further information from on-going studies on the cost-effectiveness of different screening approaches in preventing cervical cancer will be useful in formulating public health policies to guide the organization of population-based screening programmes in developing countries. The large body of research findings and managerial guidelines should be taken into account while reorganizing existing inefficient screening programmes and when considering new initiatives in low- and medium-resource settings.

Scientific Symposium
Molecular targeting in radiotherapy

30 INVITED

Epidermal growth factor receptor (EGFR) inhibitors and radiotherapy

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Background: Inhibition of the EGFR in combination with radiation is a rapidly evolving field of preclinical and clinical cancer research.
Materials and Methods: Overview of experiments and clinical studies
Results: Overexpression of the EGFR correlates with increased risk of local failure after radiotherapy. Inhibition of the EGFR by tyrosine kinase inhibitors (TKI) or monoclononal antibodies (mAb) decreases the proliferation rate of tumor cells in vitro and, in some tumor cell lines, increases cellular radiosensitivity. In tumor models in vivo regression and growth delay is generally improved by combined treatment compared to irradiation alone. In some experiments (using mAb) this translates into increased local tumor control. It could be shown that decreased repopulation of clonogenic cells and improved reoxygenation during fractionated radiotherapy contribute importantly to this effect. Several phase I and II clinical studies and one large randomised trial in head and neck cancer indicate clinical effectiveness of the combined approach.
Conclusions: Molecular targeting of the EGFR combined with radiotherapy has demonstrated effectiveness on all steps of the translational research chain. Considerable heterogeneity exists between different tumors and between different substances available calling for further mechanistic studies and development of predictive tests.

31 INVITED

Farnesyltransferase inhibitors as radiation sensitizers

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Alterations in expression or activation of signal transduction pathways are hallmarks of cancer. Additionally some of these changes are associated with alterations in drug or radiation sensitivity. Ras was the first signal transduction component shown to increase radiation resistance. Upstream and downstream pathways from Ras could thus be targets for manipulation of radiosensitivity. EGFR expression and Akt phosphorylation have also been associated with the response to radiation. Retrospective studies evaluating EGFR and Akt in patients treated with multimodality therapy found a significant association between EGFR expression or phosphorylation of Akt and treatment failure. Moreover, these data are strengthened by in vitro and in vivo studies from a large number of labs showing that inhibition of EGFR, Ras, PI3K, and Akt radiosensitized cancer cell lines.
A number of early clinical trials have now looked at signal transduction inhibitors in cancer treatment, either as single agents or in combination with chemotherapy, radiation or combined modality. Results from several of these trials will be discussed to suggest that EGFR, Ras and PI3K may mediate resistance through a common pathway. In addition to EGFR and Ras, PTEN can also regulate the PI3K pathway. Identifying a common signal for EGFR, Ras, or PTEN that results in radiation resistance may uncover targets for developing molecular based radiosensitization protocols for tumors resistant to radiation and thus improve local control.

32 INVITED

Cyclooxygenase-2 inhibitors in radiation therapy

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Dysregulation of many signaling pathways are involved in tumor development and confer resitance to standard cancer treatment. Cyclooxygenase (COX) enzymes are involved in the transformation of arachidonic acid into prostaglangines. Two isoforms of COX exist: COX-1 is thought to be the constituent isoform involved in homeostasis of normal cell functioning and COX-2 can be induced by cytokines, growth factors, and tumor promoters. Cyclooxygenase 2 (COX-2) is often overexpressed in premalignant and malignant states and overexpression is often asociated with poor clinical outcome. COX-2 derived prostaglandin participate in carcinogenesis, inflammation, apoptosis inhibition, metastasis, invasion and angiogenesis. COX-2-derived PGs have been shown to protect cells from radiation damage. There is a growing interest in the potential use of select COX-2 inhibitors in combination with chemotherapy or radiation therapy. Selective COX-2 inhibition enhances tumor response to ionizing radiation in preclinical studies both *in vivo* and *in vitro*. This increase in tumor radiation response occurs through a direct increase in tumor intrinsic radiosensitivity. The likely mechanisms involve accumulation of cells in the radiosensitive G2-M phase of the cell cycle and inhibition of repair from sublethal radiation damage. Irradiation can elevate intratumoral levels of COX-2 protein and its products, particularly prostaglandin E(2) [PGE(2)]. The increase in tumor COX-2 levels post irradiation occurs at the mRNA level, this phenomenon is blocked by COX-2 inhibitors. Inhibition of COX-2 activity or neutralization of PGE(2) activity enhances radiation response even in tumors where COX-2 expression is restricted to the tumor neovasculature. Selective COX-2 inhibitors enhance the effect of radiation on tumors that express COX-2 but not on COX-2-lacking tumors. Thus, selective COX-2 inhibitors may have potential as radiosensitizers for treatment of human cancers. Early phase I trials with radiation therapy showed that the toxicity profile is acceptable and randomized trials are required to assess the efficacy of such combinations.

33 INVITED

Molecular modulation of normal tissue radiation responses

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Increased understanding of molecular signaling pathways that control cell proliferation, apoptosis, differentiation and angiogenesis has opened new avenues for specific targeting of cancer therapeutic drugs. Many of these pathways are activated in tumors or inflammatory tissue but not in healthy normal tissues. The majority of research to date has been directed at increasing tumor responsiveness to therapy, *e.g.* by switching off activated cell proliferation signals or targeting specific oncogenes that confer radio-resistance or drug resistance. However, successful cancer treatments depend on avoiding serious normal tissue complications as well as increasing tumor response. There is now increasing awareness of the growing number of long-term survivors of cancer and the impact that late normal tissue radiation damage has, both on their quality of life and survival. This has stimulated efforts to investigate mechanisms and potential targets for intervention in the development of normal tissue damage.
Intervention strategies can broadly be categorized as aiming to increase the acute tolerance of mucosal tissues to radiotherapy or to inhibit the progressive development of late damage in irradiated tissues. The first approach generally employs specific growth factors, either to stimulate

mucosal repopulation and differentiation during fractionated radiotherapy, *e.g.* Keratinocyte Growth Factor, or to protect endothelial cells from early apoptosis, *e.g.* basic Fibroblast Growth Factor. One obvious concern with this approach is that growth factors given during radiotherapy must not have a corresponding protective effect against tumor cell kill. The alternative approach is to give intervention therapy after the completion of treatment to block some of the aberrant cell signaling initiated by radiotherapy. Examples of this are Transforming Growth Factor-beta signaling, which is associated with fibrosis, and aberrant endothelial cell signaling, *e.g.* decreased production of ADPase and thrombomodulin and increased expression of Protease Activator Receptor 1, which creates a pro-inflammatory, pro-thrombotic environment associated with many types of late radiation injury. Examples of specific molecular modulation of normal tissue radiation injury will be discussed in relation to their potential to increase the therapeutic benefit of clinical radiotherapy.

Scientific Symposium
RNAi – the new tool in cancer

34 INVITED

RNA interference and cancer: will RNA interference help to cure cancer?

A. Harel-Bellan, I. Naguibneva, M. Ameyar, A. Polesskaya, S. Ait-Si-Ali, R. Groisman, M. Souidi. *Institut André Lwoff, UPR 9079 CNRS, Villejuif France*

As an introduction to the session, this talk will describe the basic principles of RNA interference (RNAi), and review how RNAi can be used to understand, and maybe cure, cancer.
The first part will describe the RNA interference process, and how it can be (and, in fact, has already been) used to explore all pathways involved in cell fate control and in oncogenesis. Special emphasis will be put on high-throughput or genome-wide assays. This part will be illustrated with a review of the literature.
The involvement of RNA interference pathways themselves in Cancer will also be discussed, with, in particular, the implication of microRNAs in the control of mammalian cell fate and in oncogenesis. This part will be illustrated by work from the author's lab as well as by work published by others.
The second part will address the potentiality of interfering RNAs as therapeutical tools to fight cancer, and will discuss essential questions that need to addressed before we can envision such an application.
In conclusion, a naive biologist's view of future cancer treatments will be proposed to open the discussion.

35 INVITED

Functional genetic screens identify oncogenic microRNAs

R. Agami, M. Voorhoeve, C. Le Sage, R. Nagel, E. Zlotorynsky, M. Schrier, J. van Duijse. *The Netherlands Cancer Institute, Department of Tumor Biology, Amsterdam, The Netherlands*

We used functional genetic screens to identify microRNAs (miRNAs) with oncogenic potential. miRNAs have emerged in recent years as exiting new effectors of gene regulation from nematodes to man and from stem cell biology to cancer development. The number of predicted (up to a thousand) and verified (around 300) human miRNAs is still expanding. However, very few of them have been functionally annotated, partly because of the lack of reliable and concurring in silico target prediction algorithms and partly because of a lack of proper genetic tools. To overcome this gap, we created a library of vectors expressing the majority of known human miRNAs by cloning a genomic region consisting of each miRNA precursor behind a CMV promoter in a retroviral vector. Both in transient transfections and retroviral transduction, these constructs were shown to express functional miRNAs. In addition, to facilitate the identification of miRNAs that confer cellular growth advantage or disadvantage, DNA fragments, corresponding to the miRNA expression constructs, were spotted on DNA-array slides. We used the library and DNA-arrays to identify miRNAs that can protect cells from oncogenic stress. We transduced primary human cells with the miRNA library and subsequently with a retrovirus encoding oncogenic Ras or a control virus. Primary human cells stop replicating following oncogenic signals, a response that depends on intact p53 pathway and termed premature senescence. After propagating the cells for three weeks, genomic DNA was isolated and the population of miRNA inserts was compared between stressed an unstressed cells. This way we identified three miRNAs that were enriched in the RASV12 expressing population. We confirmed their activity using various growth protocols and an acidic beta-gal staining (marker for senescent cells). Furthermore, the mechanism of action of these miRNAs and their contribution to cancer development in humans will be discussed and elucidated.
See also: *http://www.nki.nl/nkidep/agamil*

36 INVITED

New technologies for RNAi-based treatment optimization

O. Kallioniemi. *Medical Biotechnology, VTT Technical Research Centre of Finland and University of Turku, Turku, Finland*

Our aim is to identify new molecular targets and mechanisms for therapeutic intervention in cancer. To achieve this aim, we develop and apply multiple high-throughput technologies including molecular profiling, RNAi-based functional screening as well as rapid clinical validation tools. Data integration from these technology platforms is applied to facilitate interpretation and prioritization of the findings.
The molecular profiling of DNA-, RNA- or protein expression patterns in samples from cancer patients is not sufficient for implicating these molecules or molecular mechanisms as therapeutic targets. It is also necessary to generate functional information on such genes and pathways. Towards this aim, we have developed a high-throughput screening (HTS) system which is composed of a robotic, automated platform for the analysis of up to 20,000 functional experiments with living cells at a time using the 384-well microplate format. Cells are dispensed into culture wells, exposed to siRNAs or small molecule compounds, incubated for 1–3 days, washed, and stained with phenotype-specific markers for cell growth, cell cycle distribution or induction of apoptosis. The results are read by plate readers or cell cytometers.
Functional studies with large RNAi libraries (e.g. 1000–10,000 siRNAs) have implicated genes whose targeting by RNAi is lethal to specific cancer types, such as breast cancer. Integration of such functional RNAi data with gene expression and aCGH data has enabled us to identify genes that are targets of genetic alterations and whose expression is required for the maintenance of the malignant phenotype. Such genes represent attractive candidate drug targets. Furthermore, we are combining RNAi screening with drug and compound screening, to identify genes that are conferring resistance/sensitivity to an existing compound, or to identify novel compounds that are effective against cells that are lacking functions of specific tumor suppressor gene or other critical genes.
Taken together, these multiple RNAi strategies should facilitate development of novel therapeutic approaches for cancer.

37 INVITED

mRNA translational control of gene expression

B.G. Wouters. *University of Maastricht, Radiation Oncology, Maastricht, The Netherlands*

Cancer can be considered a disease that arises from a series of genetic changes that alter gene expression patterns within cells. This is supported by the fact that the primary function of many oncogenes and tumor suppressor genes is to regulate gene expression. In addition to cancer-associated genetic changes, the unique tumor microenvironment can elicit further variations in gene expression that influence patient prognosis. It is thus crucial to characterize the changes in gene expression that occur within tumors, as well as their underlying mechanistic basis. Although gene expression is controlled at many different levels, research has focused principally on transcriptional regulation. In recent years it has become clear that several additional mechanisms are also important contributors to gene expression under various conditions. These mechanisms include the recently discovered microRNA's that silence gene expression through mRNA degradation or through inhibition of mRNA translation. The regulation of mRNA translation is also emerging as an important mechanism for regulation of protein expression and is often deregulated in tumors. We have shown that tumor hypoxia causes a rapid and sustained inhibition of protein synthesis at the initiation step of mRNA translation. This inhibition is controlled by (at least) two different molecular mechanisms with different activation kinetics. The early phase of translation inhibition is mediated in large part by phosphorylation of the S51 residue of eukaryotic initiation factor eIF2α. Phosphorylation occurs as a result of the activation of an evolutionarily conserved pathway termed the unfolded protein response. Prolonged hypoxia independently activates a second pathway that leads to inhibition of the mRNA cap-binding complex eIF4F. eIF4F is necessary for cap-dependent translation, and its dissociation during hypoxia correlates with the dephosphorylation and activation of the negative regulator of eIF4F assembly, 4EBP1. Although each of these two distinct pathways inhibit overall mRNA translation, they also promote the translation of a subset of genes. We were able to identify a novel translational contribution to the expression of a number of hypoxia regulated genes. These included HIF-1 target genes like CAIX, transcriptional regulators like ATF-4 and CHOP as well as translational regulators like GADD34. As predicted, the kinetics