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

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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

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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