DISSECTING CANCER CELL SIGNALLING NETWORKS AND IDENTIFYING THERAPEUTIC CANCER TARGETS

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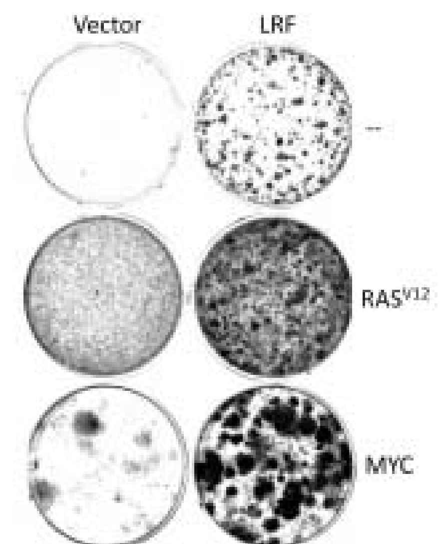
Are there any intrinsic cellular mechanisms that protect us against cancer? How can we dissect tumor-suppressing genetic networks? How can we effectively identify novel cancer genes? Can we use our laboratory results to make a difference in the clinic? For example, how can we identify novel and specific drug targets? In a nutshell, these are the fundamental as well as clinically relevant questions that we are taking up in the Peeper laboratory, which has two main focuses. First, we are dedicated to dissecting signaling mechanisms that protect mammalian cells against oncogenic transformation, aiming to increase our understanding of the molecular principles of cancer. Second, we are determined to identify novel and specific therapeutic targets as well as anticancer drugs.
Both aims are based on the premises that non-malignant cells require multiple (epi-)genetic alterations to transform into metastatic tumor cells, and that tumor cells, in turn, become dependent on activated oncogenes but also several normal genes for their survival and proliferation. To achieve these goals, we make use of advanced techniques, including screens with 100.000-vector RNAi libraries and next-generation sequencing, but we also use classical biochemical and genetic approaches.
While we are studying a diverse array of tumor types, there is a particular focus on melanoma (a highly aggressive skin cancer) and metastatic breast cancer.
At the cellular level, we have a special interest in several cancer-relevant events: (*i*) override of Oncogene-Induced cellular Senescence ('OIS', a potent tumor suppressor mechanism limiting the proliferative capacity of incipient cancer cells); (*ii*) suppression of 'anoikis' (apoptosis resulting from lack of adhesion, suppression of which may contribute to metastasis); and (*iii*) (non-)oncogene addiction (in the context of 'Synthetic Lethality' screens). Our results over the past decade demonstrate that these approaches, together, lead to the identification of signaling networks of gene products, deregulation of which allows for tumorigenesis and metastasis. Our more recent results indicate that among these, several factors exist that can be exploited for therapeutic intervention of cancer.

Ongoing

I. Breast cancer metastasis: mechanism and drug target identification

Metastasis commonly underlies the malignancy of cancers, representing the principal cause of cancer-treatment failure and patient death. Tumor spread and seeding are prevented by several physiologic barriers, including 'anoikis': apoptosis resulting from lack of adhesion. Indeed, acquiring resistance to anoikis has been proposed to represent a general prerequisite for survival of metastases during circulation. Based on this cancer hallmark, and with the help of a genome-wide functional screen, we previously identified TrkB, a neurotrophic tyrosine kinase receptor, as a potent suppressor of anoikis. Whereas non-malignant intestinal epithelial cells underwent caspase-associated anoikis in vitro, this was completely prevented by active TrkB, allowing formation of spheroid aggregates growing in suspension. Mice readily cleared parental cells, but TrkB-expressing cells formed metastases in several organs. We are currently exploring what the role is of TrkB in metastasizing tumors and whether it can serve as a therapeutic target. We have also begun to identify downstream signaling targets of this receptor. We found several transcription factors, that is, Twist, Zeb1 and Snail, whose RNAi-mediated silencing strongly suppressed the metastatic potential of tumor cells.
Further taking advantage of this experimental system, we have recently discovered a novel and critical mediator of breast cancer metastasis, the Fra-1 transcription factor. Fra-1 depletion from human breast cancer cells reduced their metastatic potential by >3 orders of magnitude. Further, by analyzing the expression of a set of Fra-1 target genes we can make accurate predictions on the clinical outcome of breast cancer. We are currently using this so-called prognostic Fra-1 signature to screen for factors that may be amenable to targeted inhibition. Eventually, this should lead to combined diagnosis/treatment options for the patient.



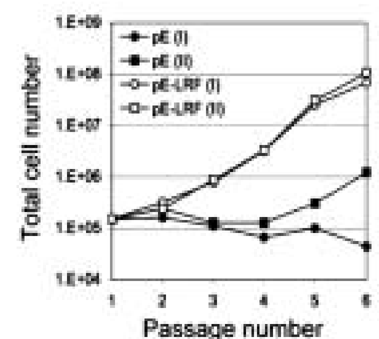


Figure 3: A genome-wide screen for oncogenes that bypass OIS identifies LRF. Shown are proliferation assays demonstrating the oncogenic activity of LRF when essayed alone or in conjunction with other oncogenes like RAS and MYC. Below is a quantification of this effect.

Publications

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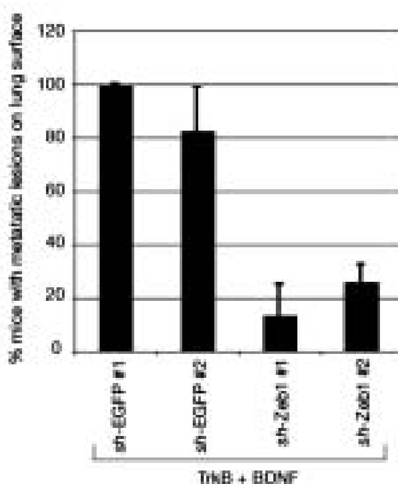

Figure 4: Identification of Zeb1 as a critical metastasis gene. Shown is the effect of RNAi-mediated depletion of Zeb1 in TrkB-expressing cancer cells: silencing of Zeb1 (with two independent vectors) strongly suppresses the ability of tumor cells to produce metastases in the lung, relative to control vectors.

II. Oncogene-Induced cellular Senescence (OIS): mechanism and drug target identification

We are identifying novel OIS pathways whose deregulation contributes to cancer in general and melanoma in particular. Also in this setting, we are combining function-based, genome-wide screens with classical molecular biological approaches. For example, we study the genetic basis for malignant progression of benign moles (nevi) to malignant melanoma. We, in a longstanding collaboration with prof. Wolter Mooi (VUmc), were the first to discover that moles display many of the classical hallmarks of OIS *in vivo*. In subsequent investigations, using a combination of genetic profiling and laser capture microdissection of compound clinical nevus-melanoma specimens, we have identified a mechanism by which nevi can escape from senescence, thereby progressing to melanoma. These results may have clinical implications, an interesting possibility that we are currently pursuing.
We have also designed several functional genomic screens with retroviral and lentiviral cDNA expression and RNA interference libraries to identify OIS escape mechanisms. For example, gene-expression microarray expression analysis of senescent cells and their 'escapers' revealed various signaling networks that are induced specifically in $BRAF^{E600}$-expressing, senescent human cells. Among the top outliers were interleukins 6 (IL-6) and IL-8. RNAi-mediated knockdown of either abrogated the senescence response. C/EBPb was identified as an OIS integrator, acting with IL-6 to amplify the activation of the inflammatory network. In collaboration with prof. Lucien Aarden (Sanquin) we found that IL-6 has antagonistic functions in OIS and proliferation, as a function of the genetic context. And together with W. Mooi, we observed that in human colon adenomas, IL-8 specifically co-localized with arrested, $p16^{INK4A}$-positive epithelium. Our results suggest a model in which the antagonistic functions of IL-6 contribute to connect senescence with an inflammatory phenotype and cancer. We propose that the dozens of cytokines that are being secreted by senescent cells serve to allow for communication between them and their microenvironment. Hence, we termed this the *Senescence-Messaging Secretome*, or SMS.
We have also recently discovered a candidate melanoma susceptibility gene, *TSC22D1*. Only the short *TSC22D1* transcript was upregulated by the melanoma driver $BRAF^{E600}$, whereas the abundance of the large protein variant was suppressed by proteolytic degradation. The TSC22D1 protein variants, in complex with their dimerization partner THG1, exerted opposing functions, as selective depletion of the short form, or conversely, overexpression of the large variant, resulted in abrogation of OIS. This was accompanied by the suppression of several inflammatory factors and $p15^{INK4B}$, with TSC22D1 acting as a critical effector of C/EBPb. These results demonstrate that the differential regulation of antagonistic TSC22D1 variants is required for the establishment of OIS and suggest a contribution of TSC22 family members to the progression of $BRAF^{E600}$-driven neoplasia.

III. Screening for novel therapeutic targets in melanoma

We have set up several screens to identify novel therapeutic targets for melanoma. Cancer cells commonly depend on activated oncogenes, as well as certain non-oncogenes, for their proliferation and/or survival, a phenomenon known as (non-) oncogene addiction. In that context, exploiting the phenomenon of synthetic lethality (SL) as a mean of therapeutic intervention is gaining increasing interest. For example, set out to screen for synthetic lethal partners of $BRAF^{E600}$. We performed an siRNA screen using the Dharmacon kinome siRNA library that contains 779 smart pool siRNAs targeting all human RNAs encoding kinases and kinase-associated proteins, in a single well format. 384-well plates were imaged by high-throughput fluorescence microscopy to determine the cell count for each well. This screen yielded a list of 61 genes whose knockdown resulted in selective toxicity to $BRAF^{E600}$-expressing cells, which are currently being validated.
Along these lines, we are setting up several additional screens, both *in vitro* and *in vivo*, to find genes that are essential for tumor progression. For this purpose, we make use of large-scale RNAi libraries and massive parallel sequencing. Together, these approaches should lead to the identification of novel therapeutic targets for cancer, and melanoma in particular.