and density of the adhesive ligand, surface topography, texture, rigidity and more. I will also describe specific siRNA screens, which were conducted to functionally map genes that are playing key roles in the formation of focal adhesion and driving cell migration. Specific attention will be focused on the molecular diversity of cancer cells, and difference in the tumorigenic behavior of cancer cell sub-populations differing in their adhesive behavior.

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DNA repair and genomic instability

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DNA damage and repair: from premature aging and cancer to longevity

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Genome stability mechanisms protect our DNA from damage by exogenous agents (e.g. UV, X-rays, chemicals) and from endogenous metabolism (ROS, lipid peroxidation). One of the most versatile DNA repair systems is nucleotide excision repair (NER), which removes a wide class of helix-distorting lesions. Two sub-pathways exist. Global genome NER operates genome-wide and prevents mainly mutations. Transcription-coupled repair removes damage that obstructs transcription, counteracting cytotoxic effects of DNA injury. Photosensitive inherited NER syndromes include xeroderma pigmentosum (XP, pigmentation abnormalities and high skin cancer predisposition) and the severe neuro-developmental conditions Cockayne syndrome (CS) and trichothiodystrophy (TTD).

Mutations in NER helicases XPB and XPD are associated with all three disorders. XPDTTD mice demonstrated that TTD is in fact a premature ageing syndrome. XPDXP/CS mutant mice are highly predisposed to cancer, but also display premature ageing, demonstrating that both phenotypes can co-exist. Different single and double repair mutants exhibit premature aging features limiting life span ranging from 15 months to 4 weeks depending on the severity of the repair defect. The correlation between severity of compromised repair and rate of onset and severity of the clinical ageing manifestations provides strong arguments for the DNA damage theory of ageing. Conditional mutants in which dramatic aging occurs only in e.g. the brain display many signs of neurodegeneration and only mild aging features in the remainder of the body. We propose that endogenous oxidative lesions hamper transcription/replication and trigger apoptosis-senescence and ageing. Microarray, functional and physiological studies have revealed that persisting DNA damage triggers a systemic downregulation of the IGF1 somatotrophic axis, causing a shift towards energy storage rather than energy production explaining the severe growth defect of the repair mutants. This 'survival' response also maximizes anti-oxidant defence. Interestingly, long-lived dwarf mice and caloric restriction exhibit a similar response. Persisting DNA damage triggers this 'survival' response in a cell autonomous manner and we provide evidence that it also implicates regulation by microRNA. These data link accumulation of DNA damage and the IGF1 control of life span.

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Chromatin signaling in DNA damage checkpoint response

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DNA damage repair and checkpoint proteins, chromatin modifying enzymes and other factors are rapidly recruited to DNA double strand breaks, forming a specialized chromatin domain. In metazoan cells, DNA damage foci likely persist until repair is complete and blocking foci formation sensitizes cells to irradiation. While the assembly, components, and functional significance of DNA damage foci remain to be fully defined, an attractive model is that foci facilitate DNA repair but also amplify DNA damage signaling. Via propagation of chromatin modifications and protein assembly over megabases of chromosomal DNA, even individual DNA strand breaks can induce apoptosis or delay cell cycle progression to allow repair and prevent aneuploidy.

Our work has been directed at examining the kinetics and molecular determinants of protein localization to double strand breaks and toward elucidating the functional consequences of disrupting protein recruitment. We are combining imaging and proteomic analysis of DNA damage foci in cancer cells using fluorescent protein fusions to the checkpoint signaling protein 53BP1. The rapid relocalization of GFP-53BP1 has facilitated kinetic analysis of DNA damage foci in living cells and identification of new protein components. We are also developing this approach to track DNA damage and repair in tumor xenograft models treated with radiation.

Taking advantage of the facile molecular genetics in yeast, we have identified determinants of activation of the 53BP1 ortholog Rad9 in G1, S phase and G2/M. In G1, phosphorylation of nucleosomal histone H2A adjacent to break sites by the ATM homolog Tel1 promotes Rad9 recruitment and checkpoint activation. Our data support combinatorial binding to modified chromatin, where tudor domains tether Rad9 to Dot1-methylated histone H3 while BRCT domains recognize phosphorylated H2A. After ionizing radiation in G1 or S phase or uncapping of telomeres in G2/M, mutations blocking signaling via H3 and H2A modifications impair phosphorylation of Rad9, prevent activation of the signal transducing kinase Rad53 and diminish checkpoint response. By contrast, defective Rfa1 single strand binding protein has little or no effect. These data suggest chromatin signaling may be necessary and sufficient for checkpoint initiation, independent of formation of single strand DNA. However, checkpoint persistence requires other factors. After irradiation in G1, Pho85 CDK activity is limiting for checkpoint recovery and S phase onset, while prolonged mitotic arrest after irradiation of nocodazole-arrested cells is independent of H3 and H2A modifications, exposing roles for the Cdc28 CDK, single strand DNA, spindle checkpoint proteins, and/or other factors.

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Mechanisms controlling the integrity of replicating chromosomes

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The checkpoint response and the SUMO-pathway counteract abnormal transitions at replication forks preventing the accumulation of reversed forks and cruciform recombination derivatives resembling hemicatenanes. Although the final targets of these regulatory processes are still unknown, the Sgs1 RecQ helicase and the Top3 topoisomerase have been implicated in the SUMO sub-pathway protecting damaged replication forks. We have investigated whether and how Top2 topoisomerase protect the integrity of replication forks. Our results indicate Top2 counteract torsional stress and sister chromatid entanglement at the forks, thus preventing the diffusion of topological changes along large chromosomal regions, abnormal chromosome transitions, DNA damage checkpoint activation and chromosome breakage during segregation. Altogether our results suggest that Top2 coordinates replication termination and S phase transcription.

We have also analyzed the dynamics of replication forks encountering a double strand break and we have unmasked a role for the Tel1-mediated checkpoint in preventing fork collapse at DNA breaks.

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An oncogene-induced DNA replication stress model for human cancer development

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Background: DNA damage checkpoint genes, such as p53, are frequently mutated in human cancer, but the selective pressure for their inactivation remains elusive. Further, most human cancers have chromosomal instability, but the genes whose mutation leads to this type of genomic instability have also remained elusive. We hypothesized that the presence of DNA double-strand breaks (DSBs) in cancer cells could explain both the presence of p53 mutations and the genomic instability.

Materials and Methods: We analysed a panel of human precancerous and cancerous lesions for the presence of DNA damage response markers using immunohistochemistry and for the presence of genomic instability by loss-of-heterozygosity analysis.

Results: In a panel of lung hyperplasias, all of which retained wild-type p53 genes, we found signs of a DNA damage response, including histone H2AX and Chk2 phosphorylation, p53 accumulation, focal staining of 53BP1 and apoptosis or senescence. Progression to carcinoma was associated with p53, 53BP1 or Chk2 inactivation and suppression of

apoptosis and senescence. A DNA damage response was also observed in dysplastic nevi and in human skin xenografts, in which hyperplasia was induced by overexpression of growth factors. Both lung and experimentally-induced skin hyperplasias showed allelic imbalance at loci (common fragile sites) that are prone to formation of DNA DSBs when DNA replication is compromised. Further, in various model systems, oncogene overexpression led to stalling and collapse of DNA replication forks and generation of DNA DSBs.

Conclusion: From its earliest stages, cancer development is associated with DNA replication stress, which leads to DNA double-strand breaks, genomic instability and selective pressure for p53 mutations.

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Genetic epidemiology / Whole genome scan

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Identifying new breast cancer genes through international consortia

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Breast cancer, like other common cancers, tends to cluster in families. This clustering is predominantly genetic in origin. Most of the genetic effect is probably polygenic – that is, the result of the combined action of many genetic variants of small effect. We have recently completed a genome-wide scan for common genetic variants that contribute to susceptibility, and have identified 5 new predisposing loci. The possible future applications of this knowledge to breast cancer detection and prevention will be discussed.

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Genome wide association studies for breast and prostate cancer susceptibility loci in the CGEMS initiative

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Three major advances have recently made possible genome-wide association studies (GWAS). First, the realization that locus-specific relative risks are usually small and thus require in order to be detected international consortia able to pool into joint analyses large numbers of patients and controls. Second the establishment of the first repertoire of the human genetic diversity by the HapMap project. Finally, the development of cost-effective techniques enabling the genotyping of a DNA on hundreds of thousand of loci in a single step. The judicious selection of half a million markers provides useful information on 80% of the estimated 7 million SNPs with minor allele frequencies higher than 0.05 present in a population of European origin. With the potential to explore a large fraction of the genome, the initial requirement of functional hypotheses to perform association studies becomes unnecessary. Recognizing the promises of this new approach, the NCI has launched the Cancer Genetic Markers of Susceptibility (CGEMS) initiative which aims at providing to the scientific community the results of GWAS for breast and prostate cancer.

The planned strategy involves for each tumor type three stages. In the first stage, about 1100 cases and 1100 controls nested in a prospective cohort are typed on 500,000 markers. The statistical analysis of genotypic data identifies a set of 25,000 SNPs with p-value for association lower than approximately 0.05 and with low pair-wise correlation among them (r^2< 0.8). In the second stage, these SNPs are typed on about 4,000 cancer cases and 4,000 controls. The subsequent analysis identifies about 150 chromosomal regions, each containing at least one SNP with a p-value smaller than 10^-3. SNPs in these regions are taken to the third stage which involves the genotyping of an additional set of 5000 cases and 5,000 controls. At stages 2 and 3, regions with convincing indication of being truly associated (low p-value in CGEMS and/or reported by others to be associated) are investigated with a dense set of markers. In order to investigate a total of over 40,000 DNAs, collaborations involving multiple European and American groups were established.

The first stage has been completed for both tumor types. The results have been posted on a public web site in October 2006 and April 2007 for the prostate study and in May 2007 for the breast study. They provide genotype counts and and p-values under various statistical models for over 500,000 SNPs. The second stage for the prostate study has been posted in March 2008 and provides follow-up data for 27,000 SNPs The second stage for the breast study will be released in the second half of 2008. Analysis of the second stage of the prostate study revealed 7 loci with p-values less than 2.5 10^-6 including three previously known loci and 4 new ones. In addition, 9 new loci showed suggestive association ($p < 2.5\ 10^{-5}$). The best significance ($p<7.4\ 10^{-13}$) was observed for MSMB, which encodes a primary constituent of semen and is proposed prostate cancer biomarker.

Combined with results from other published GWAS, fifteen loci have been convincingly associated with prostate cancer susceptibility. The risk-allele frequency may reach 0.85, indicating lack of natural counterselection. Most per allele odds ratios are small, typically 1.2. Importantly, to date, none of the functional polymorphisms have yet been unambiguously identified for any of the loci, and for many of them the relevant functional gene(s) remain(s) elusive. Knowledge of these loci provides unique original leads for further investigation in the mechanism of tumorigenesis. We will soon know if similar conclusions may be drawn from the CGEMS breast cancer study. The use of this new information to predict individual cancer risk for improved patient management requires validation by large studies, as little is known on the interaction of multiple risk-alleles co-existing in the same individual.

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Issues and opportunities in family-based designs for young-onset cancers

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Conditions with onset early in life, such as childhood cancers, can have complex etiologies, because both genetic and environmental factors can contribute to risk, and also because both the maternal and the fetal genomes can play a role. Classical case-control analysis exploring effects of inherited genetic variants is vulnerable to confounding by the maternal genotype, which can be causally related to the outcome (prenatal effects) and is certainly causally related to the genotype of the offspring. Consider a "triad" design, where one studies offspring with cancer together with their mothers and fathers. Under a simplifying assumption of genetic mating symmetry, a log-linear analysis (i.e. Poisson regression) can efficiently disentangle effects of inherited variants from prenatal effects mediated through the maternal genotype. Taking advantage of the mathematical distortions produced by over-transmissions of risk-related variant alleles to affected offspring, one can estimate effects of autosomal fetal genetic variants, with full robustness against bias due to population stratification or failure of Hardy-Weinberg equilibrium in the source population. One can also take advantage of the asymmetries induced between the maternal and paternal genotypes to identify maternally-mediated effects. Using this design, one can thus efficiently distinguish effects that work through the fetal genes from those that work through expression of maternal genes during gestation. No inheritance model needs to be assumed (e.g. dominant or recessive) and families with a missing parent or a deceased offspring can also be included. Multiplicative models can be assessed for examining joint effects with environmental factors. A limitation of the triad approach involves the fact that although main effects of genetic variants and multiplicative interactions with environmental effects can both be studied, one cannot assess main effects for exposures. A hybrid approach extends the design by including the parents of population-based controls, and provides greatly improved power and flexibility for inference related to joint effects.

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Detecting new genes for tobacco-related cancers - genomewide association study of lung cancer

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Lung cancer is the most common cause of cancer death worldwide with over 1 million cases annually. While a heritable component for lung cancer