Department of Gynecology and Obstetrics

Head: Wolfgang Janni

Division of Gynecological Oncology

Head: Lisa Wiesmüller

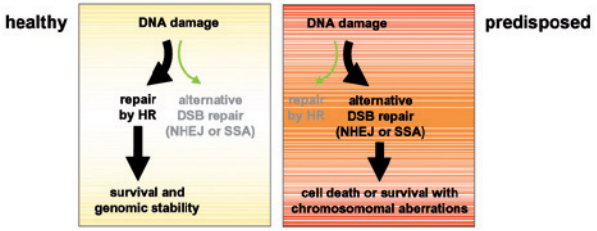
DNA Repair, Tumor Suppression and the Aging Process: In Search of Mechanisms and Markers

Failure to repair DNA double-strand breaks (DSBs) causes immunodeficiencies, chromosome instability syndromes and progeria. Error-prone repair causes genomic instabilities that accelerate the multistep process of tumorigenesis. We developed assay systems for the analysis of all DSB repair pathways in immortalized and primary cells from different organs. The power of pathway-specific testing to detect even subtle DSB repair deficiencies was documented by testing cells derived from a series of *Ataxia telangiectasia*, Nijmegen breakage syndrome and Fanconi anemia patients as well as from a collection of breast cancer patients with mutations in *BRCA1*, *BRCA2* or *CHEK2*. Having identified a phenotypic signature that captures various defects resulting from breast-cancer-predisposing alterations, we performed the first case-control study for prospective evaluation of this potential biomarker in peripheral blood lymphocytes. The results showed that error-prone DSB repair activities were elevated in women with familial risk (Fig. 1) and in breast cancer patients of young age. Importantly, the risk-specific signature also captures synthetic lethal interactions with inhibition of PARP1, which has become an extremely promising target for therapies selectively eliminating repair-defective tumor cells.

Combined use of DSB repair testing, genomic PCR and quantitative analysis of nuclear structures indicative for DNA lesions, repair intermediates and/or enzyme complexes elucidated particular mechanisms underlying genetic destabilization in hematopoietic malignancies such as upon expression of BCR-ABL or constitutive NF-kB activation (Fig. 2). Further projects are aimed at the identification of novel disease-causing genes, synergies between cancer susceptibility and modifier genes through combination of functional testing (patient families/cohorts, mouse models) and genotyping (siRNA screen, whole exome sequencing) as well as the mechanistic characterization of the development of secondary, therapy-induced leukemias with implications for risk prediction.

Interestingly, recent literature has described the striking links between replicative senescence, telomere maintenance, aging and DSB repair. We characterized the role of and functional interactions

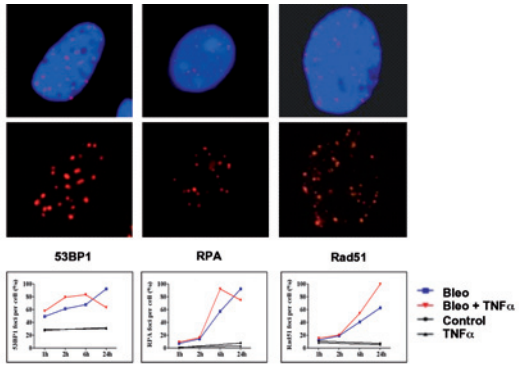
The Team:
Head of Department: W. Janni
Head of Division: L. Wiesmüller
Group Leaders/Postdocs: B. Gole, D. Salles, A. Stahl, M. Uhl
PhD Students: M. Volcic, I.C. Ireno, K.J. Obermeier
Additional Members of Thesis Advisory Committees:
M. Filipic (Lubljana), S. Fulda (Ulm), H. Geiger (Ulm), P. Gierschik (Ulm), M.E. de Lima Perez Garcia (Belo Horizonte), R. Winqvist (Oulu)

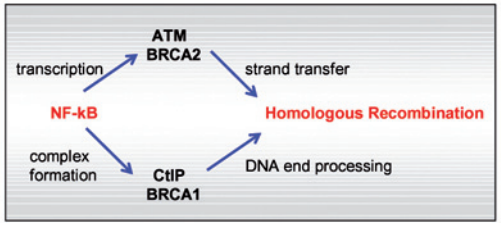


DSB repair substrate	High risk individuals n	Controls[b] n	Unit	OR[c] (95% CI)	P value[d]	Area under the ROC[e] curve
EJ-EGFP	35	144	0.45	1.71 (1.21-2.49)	0.0034	0.66
Δ-EGFP/3'EGFP	18	94	0.33	2.61 (1.57-4.84)	0.0007	0.71
HR-EGFP/3'EGFP	14	74	0.39	4.03 (1.86-11.92)	0.0045	0.72

Predictive power of error-prone DSB repair activities for discrimination between high-risk individuals versus controls. Our team previously showed that a shift from error-free HR to error-prone repair pathways using the DNA substrates EJ-EGFP, Δ-EGFP/3'EGFP, and HR-EGFP/3'EGFP can be detected in lymphoblastoid cells from individuals with breast-cancer-predisposing mutations. Consequently, we performed the first case-control study for prospective evaluation of this potential biomarker in peripheral blood lymphocytes from 35 individuals of high breast and ovarian cancer risk families, 175 sporadic breast cancer patients, and 245 healthy donors. We found increases of error-prone repair in women with familial risk versus controls particularly when applying substrate HR-EGFP/3'EGFP (Odds Ratio 4.05).

between different aging-related proteins in DSB repair such as ATM, Fanconi anemia gene products, SIRT1, p53 and PARP1. The challenge is now to understand the details of how DSB repair is regulated during the aging process in differentiated versus stem cells. Regarding DSB repair and its accuracy in hematopoietic stem cells, data are scarce and in part contradictory. Regarding DNA repair as a function of age in human beings, first data sets suggest that age exacerbates chromosome damage. However, the underlying mechanisms have remained enigmatic and emphasize the need for systematic investigations. Taken together, the purpose of our research is to understand the molecular details of DNA damage response mechanisms and their deregulation during aging, carcinogenesis and in chromosome instability syndromes. Our ultimate goal is to develop biomarkers to monitor/detect age-related processes, cancer risk, and therapeutic responsiveness.





Role of NFκB in DSB repair. DSBs were induced by exposure to the radiomimetic drug bleomycin (bleo) alone or in combination with the NFκB activator TNFα. To visualize various stages of DSB repair, cells were processed for immunolabeling of 53BP1 indicative of DSBs, for RPA indicative of single-stranded DNA, and Rad51 indicative of the HR machinery. Focal signals in the DAPI-stained nucleus were analyzed on an Olympus BX51 epifluorescence microscope and quantified by AnalySIS software including the mFIP module. From these and further data published in Volcic et al. 2012, a model of NFκB-dependent DSB repair regulation was proposed.

Ulm University
Department of Gynecology and Obstetrics/
Division of Gynecological Oncology
Prof. Dr. Lisa Wiesmüller
Prittwitzstraße 43
89070 Ulm, Germany
Tel. +49 (0)731 500 58800
Fax +49 (0)731 500 58504
lisa.wiesmueller@uni-ulm.de
www.uni-ulm.de/klinik/ufk/cms/html/forschung_gyn-onko.htm

Selected Publications:

- *Böhringer M*, Obermeier K*, Griner N, Waldraff D,Dickinson E, Eirich K, Schindler D, Hagen M, JerryDJ*, Wiesmüller L* (2013): siRNA screening identifies differences in the Fanconi anemia pathway in BALB/c-Trp53+/- with susceptibility versus C57BL/6-Trp53+/- mice with resistance to mammary tumors. Oncogene, in press.*
- *Keimling M*, Deniz M*, Varga D, Stahl A, Schrezenmeier H, Kreienberg R, Hoffmann I, Koenig J*, Wiesmüller L* (2012): The power of DNA double-strand break (DSB) repair testing to predict breast cancer susceptibility. FASEB J 26, 2094-2104.*
- *VolcicM, KarlS, BaumannB, Salles D, DanielP, FuldaS, Wiesmüller L (2012): NF-κB regulates DNA double-strand break repair in conjunction with BRCA1-CtIP complexes. NucleicAcids Res 40, 181-195.*
- *Keimling M*, Volcic M*, Csernok A*, Wieland B, Dörk T, Wiesmüller L (2011): Functional characterization connects individual patients' mutations in Ataxia Telangiectasia Mutated (ATM) with dysfunction of specific DNA double-strand break repair signalling pathways. FASEB J 25, 3849-3860.*
- *Salles D, Mencalha AL, Ireno IC, Wiesmüller L*, Abdelhay E* (2011): BCR-ABL stimulates mutagenic homologous DNA double-strand break repair via the DNA-end-processing factor CtIP. Carcinogenesis 32, 27-34.*
- *Gatz SA, Wiesmüller L (2006): p53 in recombination and repair. Cell Death Differ 13, 1003-1006.*

** equal contributions*