ylation of ATM and its substrates, H2AX and p53. This subsequently led to the activation of PUMA, caspases and to apoptotic cell death. Next, we used an ATM inhibitor, KU 55933, which has been shown previously to be a radio/chemo-sensitizing agent. Pretreatment of resting T cells with KU 55933 blocked phosphorylation of ATM, H2AX and p53, which, in turn, prevented PUMA expression, caspase activation and apoptosis. However, etoposide-induced DNA damage in resting T cells was not influenced by KU 55933 as revealed by the FADU assay. Altogether our results show that KU 55933 blocks DDR and apoptosis induced by etoposide in normal resting T cells. We have also shown that curcumin, a natural agent with anticancer potential, can induce apoptosis of normal resting human T cells that is not connected with DNA damage. Our results also revealed that T cell stimulation can induce DNA damage and DDR which can be the primary reason of T cell senescence *in vitro*.

SW01.S3–41

Das13 mutation in bacteriophage T4 RNase H increases its exonuclease activity

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Bacteriophage T4 RNase H, a member of FEN-1 endonuclease family, possesses both flap endo- and 5′–3′ exonuclease activities on double-stranded DNA. The mutations mapped to *rnh* gene encoding for RNase H (known also as *das* mutations) were selected as specific suppressors of DNA arrest phenotype caused by defect in *46* and/or *47* genes. Recently, it was shown that product of these genes is a nuclease complex, which is an analog of eukaryotic Mre11/Rad50. We have sequenced the phage T4*das*13 *rnh* gene and found two mutations, which lead to amino acid substitutions V43I and L242I. The analysis of known 3D structures of phage T4 RNase H has shown that both mutations do not participate in formation of the active center or DNA-binding sites. In order to determine whether these mutations affect the activity of the enzyme on dsDNA the nuclease assays were carried out. We tested both wild-type protein and three mutants (Das13 with both substitutions, and proteins with single substitutions V43I or L242I) for the nuclease activity *in vitro*. It was found that the activity of single mutant V43I and Das13 protein was almost the same and significantly increased, while the activity of L242I mutant was similar to the wild type protein. To study the impact of V43I (and Das13) substitution on endonuclease and exonuclease activity the nuclease assay on synthetic substrates was performed. It was found that exonuclease activity of V43I mutant on GAP-substrate was in 10 times higher compared to the wild type RNAse H, whereas its endonuclease activity on Y-substrate remained unchanged. In addition, in our experiments, the mutant phages, carrying V43I substitution in RNAse H were able to suppress conditionally lethal mutation in gene *47*.

Taken together, our findings suggest that the single mutation V43I is sufficient to increase exonuclease activity of phage T4 RNase H and to suppress DNA arrest phenotype caused by defect in gene *47*.

SW01.S3–42

An inter-species landscape of DNA repair proteins based on extreme metagenomes and repeated HMM profiling

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Next generation sequencing technologies made possible the discovery of numerous new microbe species in environmental samples. Perhaps it is more important to discover new genes of remarkable functions and properties. In this study, we identified proteins in DNA repair in well-known organisms (i.e. proteins in base excision repair, nucleotide excision repair, mismatch repair and DNA break repair); next we applied multiple alignments (with CLUSTAL) and then built HMMER profiles for each protein separately, across the well-researched (model- and non-model organisms); next, using public depositories of metagenomes, originating from extremely hot, extremely cold, or extreme pH environments, we identified DNA repair genes in the samples. Note, that the phylogenetic classification of the samples are not typically available.

We hypothesized, that some very special DNA repair strategies need to be applied in bacteria and archaea living in those extreme circumstances.

It is a difficult task to evaluate the results obtained from mostly unknown species. Therefore we applied again the HMMER profiling: for the identified DNA repair genes in the extreme metagenomes, we prepared new HMMER profiles (for each genes separately, subsequent to a cluster analysis); and we searched for similarities to those profiles in well-known model organisms.

We have found well known DNA repair proteins, lots of proteins with unknown functions, and also proteins with known, but different functions in the model organisms. We describe the results of this work in our presentation.

SW01.S3–43

New players in recognition of AP sites in clustered DNA damages

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DNA repair is the central mechanism to keep integrity of cell genome. One of the most abundant lesions in DNA is apurinic/apyrimidinic sites (AP) sites arising spontaneously or under genotoxic stress conditions. AP sites are unstable and cytotoxic. Attempted repair of bistranded AP sites (i.e. situated in both DNA chains) can result in formation of double-strand breaks – the most deleterious DNA lesion. One can suggest an existence of proteins that are able to specifically interact with AP sites in clustered DNA damages and regulate their processing.

DNA containing bistranded AP sites were used to trap in mammalian cell extracts proteins interacting with AP sites via Schiff-base intermediate.

Cross-linked proteins were identified by peptide mass mapping (based on MALDI-TOF-MS analysis) and immunochemical approaches.

High-mobility group box 1 and 2 proteins, abundant multifunctional non-histone chromatin proteins, were shown to more efficiently interact with bistranded AP sites than isolated ones, and in reconstituted system interfere with AP sites hydrolysis by AP endonuclease 1, the main mammalian protein cleaving AP sites.

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