Using DNA damage repair system from model organisms to study human Cancers and DNA damage repairs: A valid approach?

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1. Abstract

DNA damages left unrepaired which accumulate in course of time are one of the most important causative agents of cancer. With aging inherent DNA damage repair systems suffer a loss of efficiency to repair DNA damages. It is evident that DNA repair genes are gatekeepers of cancer and hence defective or inadequate DNA damage repair systems do play the key role in the development of cancer. Because of cancer in affects multiple organs and systems in humans, it becomes a most challenging job to model these processes in the lab. So far the cancer research has been dominated by non-vertebrate short-lived model organisms such as yeast (*S. cerevisiae*), worm *(C. elegans)* and fly *(D. melanogaster)* that has enabled the identification of remarkably conserved aging-related pathways. In spite of this, some important aspects of human cancer and disease phenotypes cannot be confidently recapitulated in invertebrate models as they are devoid of specific organs and systems that are crucial components of human cancers. Vertebrates model systems; mouse *(M. musculus)* and zebrafish *(D. rerio*) have also been utilized in cancer research. Still, experimental studies have been affected by the comparatively long life span of mice and zebrafish and their high maintenance costs particularly mice. Mouse models with accelerated onset of age-related diseases can address the cancer medical conditions in parts but they still need detailed studies to discover the role of the DNA damages which accumulate during aging and failed DNA damage repair systems in the cancer progression. All these issues related to model organisms available at present led to the need of the new vertebrate model of DNA damage repair systems that can be ultimately translated into the humans for cancer treatments and prevention.

2. Acknowledgement

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I am very happy to have the opportunity to work at this group.

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3. Introduction

Cells are continuously exposed to endogenous and exogenous agents that induce damage to the cellular macromolecules DNA, RNA and proteins (Harmean, 1992). DNA is an important molecule for the cells that carries the inherited genetic information used in growth, development, functioning and reproduction of all known living organisms (Gredilla, et al., 2012). Unlike damaged lipids and proteins, damaged DNA cannot be replaced hence DNA lesions formed can have the great impact on the genome stability (Gredilla et al., 2012). To repair such damaged DNA, DNA damage repair systems are highly conserved from human to bacteria (Gredilla et al., 2012). Impaired and insufficient DNA damage repair systems and DNA replicative mechanisms cause accumulation of the DNA damages leading to the cancers (Harmean, 1992; Jeggo et al., 2016; Hakem 2008; Bon and Lerebeke 2004) (Friedberg, 2003; Farmer et al., 2003). Hence the study of the repair systems is of utmost importance and best way available for this is animal models (Gredilla et al., 2012). Recently different animal models have been studied for the investigation of the strong and highly efficient DNA damage repair systems that can be ultimately translated into the humans for cancer treatment and prevention. In this essay, we have given the detailed account of the DNA damages, it's causative agents and different animal models that can be used to study DNA damage repair systems.

DNA damage is caused by excessive amounts of the endogenous and exogenous agents. There is a wide range of the DNA damage sources like chemicals, UV radiations from the sun, byproducts of the endogenous metabolic products (Bon and Lerebeke 2004; Helleday 2014). Aging, carcinogenesis, and mutagenesis have one causative agent in common that is DNA damage. Chemicals events like hydrolysis, exposure to the ROS and other reactive metabolites lead to DNA damage. It is observed that DNA damage caused by both endogenous (spontaneous) and exogenous (environmental) factors contribute to mutations. Endogenous metabolic processes and exogenous chemicals trigger chemical events that lead to DNA damage (De Bont & van Larebeke, 2004). Concentration and mutagenic potential to which humans are exposed in the environment are not sufficient to explain the high incidence of the cancers that have occurred actually in the population but are not due to inherited gene changes (Epe, 2002). Innate factors are also inadequate to explain these cancer conditions observed frequently. From epidemiological studies, in developed countries, 75-80% of the cancer cases have exogenous factors as one on the potential contributors with obligatory presence of endogenous causative agents (Doll and Peto, 1981; Trichopoulos et al., 1994). From different observations, it can be concluded that mutations caused by DNA damage, exogenous chemicals and endogenous metabolic processes and their products play a role in most cancers. At present, it is not clear if, DNA damage caused due to endogenous metabolic processes and their factors are further modulated by any exogenous chemical agents. Compared to exogenous DNA damage endogenous DNA damage occurs more frequently. Types of DNA damages created by normal cellular processes are similar to those of caused by environmental agents (Jackson and Loeb, 2001).

4. Types of DNA damages

4.1 Oxidative DNA damage

As the byproducts of the metabolic processes and biochemical reactions carried out spontaneously, and due to some external factors ROS are continuously generated in the cells. ROS include Superoxide O2- , hydrogen peroxide (H2O2), hydroxyl radicals (OH-) and singlet oxygen (1O2) (Iida et al., 2001; Li et al., 2002). These ROS are capable of oxidizing DNA that results in different types of DNA damage such as oxidized bases of DNA, single as well as double stranded breaks (Kono and Fidovich 1982). Because ROS are highly active, damage resulting due to them is most frequent. Oxidative DNA damage occurs when the body's natural antioxidants fail to control the production of ROS (Kono and Fidovich 1982; Tabatabaie and Floyd, 1994). ROS possess the ability to inactivate the antioxidant enzyme(Slupphaug et al., 2003, Kono and Fidovich 1982, Tabatabaie and Floyd, 1994). Productions of the ROS take place because of exogenous as well as endogenous processes. Inflammatory responses elicited by chronic infections are great generators of the ROS (Jackson and Loeb, 2001). Neutrophils produce the oxygen which is then supplied in bulk for the formation of superoxide radicals and hydrogen peroxide. These two species react and form potent hydroxyl radical. (Jackson and Loeb 2001). Formidopyrimidine adducts of adenine and guanine that is 4,6-diamino-5-formamidopyrimidine (FapyAdenine) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGuanine) are oxidatively modified bases that are abundant (Burgdorf and Carell 2002). 8-oxo-adennie and 2-hydroxy-2'-deoxyadenosine are other modified adenine bases. Oxidized base thymine glycol base pairs with the adenine and results in cytosine to thymine transition mutation (Marnett and Plastaras 2001). This mutation blocks the replication and transcription but it is the weak mutagen (Basu et al., 1989, Dianov et al., 2000). Among all, 5-OH-Cyt is most mutagenic that any other mutagen that is produced by oxidative DNA damage (Feig et al., 1994). Table 1 summarizes the measurements of the oxidative DNA adducts in human tissues. DNA lesions were observed to be in increased amount as the result of the conditions caused by oxidative DNA damage sources (Bartsch and Nair 2000) and contribute to the risk of cancer.

Table 1: Oxidative DNA damage: Adduct and Tissue they formed in (Adapted from Bont R. and Larebeke N., 2004 [endogenous DNA repair I])

Adducts	Human Tissue
5-Hydroxy-2'-deoxycytidine	Leucocytes, Lymphocytes, Bronchial epithalium
5-Hydroxy-2'-deoxyuridine	Leucocytes
5,6-Dihydroxy-5,6dihydro-2'-deoxyuridine	Leucocytes
8-Oxo-7,8-dihyro-2'-deoxyguanosine	Leucocytes, Lymphycytes, Pancreas

4.2 Endogenous alkylating mutagens

Alkylating agents are reactive methyl group donors that play role in enzymatic DNA methylation associated with the regulation of gene expression. (Holliday and Ho 1998). The most important of these mutagens is S-adenosyl methionine (SAM). SAM methylates purified DNA non-enzymatically observed in the experiments nearly identical to the extent at which it happens in vivo and generates 4000 7-methylguanine, 600 3-methyladenine and 10-30 O6-methyl-guanine residues per day in mammalian cells. DNA methylation results in a formation of the 7-Methylguanine and 3- methyladenine. 7-Methylguanine does not create any changes in coding specificity hence it is not regarded as harmful whereas destabilization of the

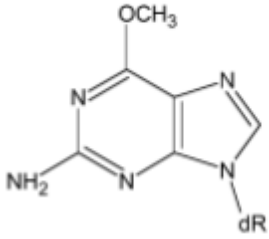

Fig. 1: O^6-methylguanine (Bont R. and Larebeke N., 2004)

glycosyl bond because of N-7 replacement on guanine generates apurinic (AP) site and that also opens imidazole ring opening of –methylguanine which causes mutation and replication stoppage respectively. (Barberella, et al., 1991, Tudek et al., 1992). 3-methyladenine is a cytotoxic DNA lesion that blocks the replication (Atamna et al., 2000). O6-methylguanine Figure 1, O4-methylthymine and O4-ethylthymine are highly mutagenic DNA lesions.

These lesions result into GC-AT and TA-CG transitions during DNA replication (Gerchman and Ludlum, 1973, Abbot and Saffhill 1979, Safill 1985, Singer et al., 1986). Cyclic adducts of the purine DNA bases are formed after reactionwith 4,5-dioxovaleric acid and 2,4 decadienal that are involved in lipid peroxidation (process by which reactive substances damage DNA by formation of the exocyclic adducts and are highly mutagenic)

Table 2: Adducts formed due to DNA alkylation and tissue in which they form (Adapted from Bont R. and Larebeke N., 2004)

Adduct	Tissue
7-Methylguanine	White Blood Cells (WBCs), WBCs of smokers with lung cancer, Lymphocytes, Granulocytes, Lung tissues, Bronchus, Pancreas, Colon mucosa.
O^6-methylguanine	Leucocytes, Liver, Lung tissue smokers
O^4-methylthymine	Liver
O^4-ethylthymine	Liver
7-(2-Hydroxyethyl)guanine	Blood cells, WBCs smokers with lung cancer, Lymphocytes
7-Alkylguanine	Leucocytes, Larynx
7-Ethyl-dGP	Lymphocytes, Lung
7-Methylguanine and 7-(2-hydroxyethyl)-guanine	Lymphocytes smokers and non-smokers

4.3 DNA hydrolysis

The glycosidic bond in DNA is formed between the bases (purine and pyrimidine) and deoxyribose (sugar group) and it is susceptible to get cleaved under some specific conditions like heating, alkylation of the bases (Lindahl, 1982). When this glycosidic bond between the DNA bases and sugar moiety get the cleaved formation of the basic site takes place. Destabilization of the glycosidic bond due to N-7 substitution on the guanine results into the generation of the mutagenic apurinic site (AP site) (Barbarella et al., 1991; Tudek et al., 1992), spontaneous depurination and to the large extent ROS produce AP sites (Nakamura et al., 2000). Most frequent endogenous lesions found in DNA are abasic sites i.e., 10,000 lesions/human cell/day (Lindahl, 1993). Purines are lost at 20 times higher rate than that of pyrimidines in DNA (Lindahl and Karlstrom, 1973).

Table 3: Quantity and tissues where of Abasic sites are found (Adapted from Bont R. and Larebeke N., 2004)

Tissue	Quantity
Cells (leucocytes)	2000 – 10 000 lesions/cell generation
Liver	8-9 lesions/10^6 nt, 50 000 – 200 000 lesions/cell
Cultured cells/leucocytes	<0.67 lesions/10^6 nt or 4000 lesions/genome

Endogenous AP sites affect the brain at the greatest extent followed by colon and heart and then liver, lung, and kidney (Nakamura and Swenberg, 1999). The remarkable fact was that the number of AP sites in human leucocytes from an old donor was about seven times that in younger donors that clearly implies the effect of aging on the DNA damage in other worlds there is more accumulation of DNA damage in old people than young (Atamna et al., 2000).

4.4 Hydrolytic deamination

Hydrolytic deamination mainly targets cytosine and 5-methylcytosine (Lindahl, 1993). DNA bases are already labile to get deaminated. Single stranded DNA is more susceptible to get deaminate compared to double-stranded DNA. Around 100 to 500 cytosines/cell/day are deaminated to uracil(Lindahl 1993). Spontaneous mutations attack 5-methylecytosine more frequently because it can deaminate 3-4 times more rapidly than cytosine (Lindahl 1979). Uracil-DNA glycosylase excises the deaminated form of cytosine; that generates a base free site that gets corrected. At this site, GT base pair formation takes place by deamination of 5-methylctyosine and hence GC to AT transitions result at the site of cytosine methylation that cause one-third of single site mutations. Single site mutation cause some inherited disease in humans (Cooper and Youssoufian, 1988). In many human cancers, same base change as described above is highly occurring in a mutated p53 gene (Rideout et al., 1990). Deamination of DNA purines is comparatively less observed phenomenon compared to deamination of the cytosine to uracil.

4.5 Exogenous DNA damage

Exogenous (Environmental) origins of DNA damage can be physical or chemical. For instance, an ionizing UV radiation and X-irradiation are examples of a physical agent with extremely high energy to excite molecular bonds. UV-irradiation caused DNA damage creates excision in the DNA and these are measured in different species (Setlow et al., 1972). Lethal and mutagenic responders from the biological systems to mutagens like UV-irradiation depend basically on a change in DNA. DNA has

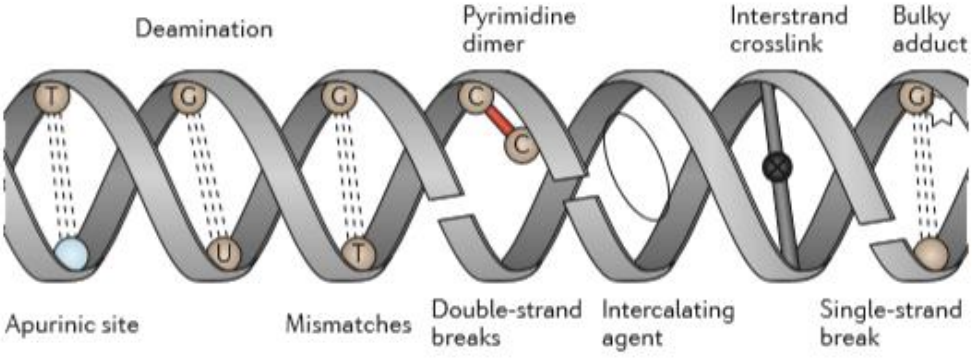

Fig.2: Overview of types of DNA damage and casative agents (Helleday et al. , 2014)

high absorption co-efficient for UV-irradiation and X-irradiation as it has large molecular weight. Composition, conformation presence of the sensitizers and protectors and energy transfer mechanisms decide the physiochemical changes that DNA will undergo when exposed to ionizing radiation. DNA damage effects on the cells like conformational changes, strand breaks, unusual crosslinking, and base damage depend on repair system capabilities to ignore, bypass and repair defects. These radiations cause covalent changes between two adjacent pyrimidine nucleotides. As a result of this event, pyrimidine dimers are formed. Chemical agents distort DNA structure by intercalating by binding covalently to the DNA in various fashions. Each different fashion causes particular mutational signature. For instance, chemotherapeutic alkylating agent cyclophosphamide and temozolomide

result in CG-AT transitions and benzo(a)pyrene diol epoxide that is the carcinogenic by-product of tobacco smoking cause GC-TA transversion. From these examples, it can be understood that depending on the exposure of different chemicals different mutations take place. As glycosidic bond is labile, base loss due exposure to various mentioned mutagens is approximately 104 bases per cell per day and this results in the formation of apurinic and apyrimidinic sites shown in figure 2. Depurination occurs more readily which makes apurinic sites more frequent present than apyrimidinic sites. Other damage types include deamination, replication errors, and formation of free radicals. Free radicals create double strand breaks. Non-ionizing ultraviolet radiation is responsible for the biochemical modifications such as the formation of pyrimidine dimers and these when left un-repaired can be mutagenic. Chemical compounds such as platinum-based compounds such as cisplatin cause bulky adducts or inter and intrastrand crosslinks. The intercalating agents like benzo[α]pyrenes, daunorubicin and actinomycin-D; DNA alkylating agents such as methyl methanesulphonate (MMS) cause DNA damage by intercalation (Helleday et al., 2014). To find out chemical associated with each kind of mutation will take a great time as patients history of exposure to various chemicals will have to be discovered in details. Some other types of chemical agents are known to cause exogenous DNA damage as described in figure 2.

5. DNA damage repair systems in humans and model organisms

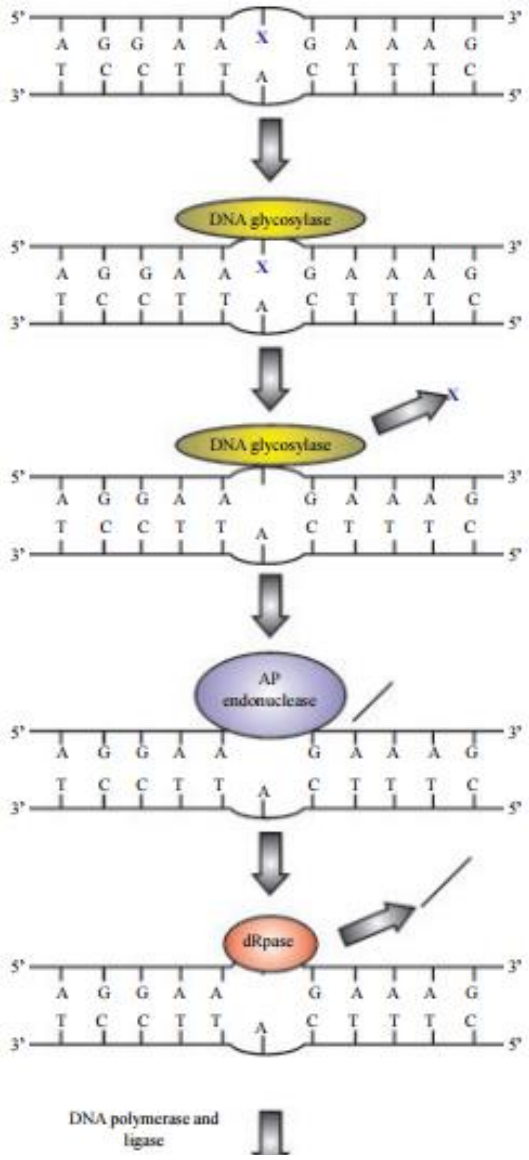

*Fig. 3: Base excision repair (BER). Damaged base is represented as **X** (paper III: Pinto L., et al., 2003)*

When bacterial and human DNA repair systems are analyzed, a large number of similarities are observed in the ways these groups of organisms repair DNA damages (Arvind et al., 1999). DNA damage repair systems are extremely important for life itself as if they are not there to make the necessary corrections when DNA damages have occurred, it will be incompatible with all life forms that exist. Therefore, metabolic patterns performing the role of safeguards against the DNA damage are conserved throughout the evolution. There are high degrees of similarities in the way that eukaryotic organisms and bacteria repair DNA damage. As the same kind of chemical reactions takes place in the genome of these organisms, similar kinds of DNA damage patterns are generated. Given this fact, it is no surprise that the biochemical pathways used to remove DNA damage are similar in eukaryotic cells and bacteria. For instance, the DNA damage repair pathway to repair generated AP sites is very similar in eukaryotic cells and bacteria. These sites are repaired after the action of AP endonucleases which catalyze incision of the DNA at AP sites and prepare DNA for subsequent excision, repair synthesis and strand sealing. Amino acids among the AP endonucleases are considerably conserved as high as 41% (Popoff et al., 1990).

Metabolic processes in the eukaryotic organisms are more complex. Compared to bacteria some proteins have multiple functions in cell cycle checkpoints, chromatin assembly, DNA replication and repair. For example, in the nucleotide excision repair pathways, five different proteins are involved in the bacteria while 15 different proteins are essential in eukaryotic organisms for the same pathway (Cleaver et al., 2009).

The major pathways involved in DNA repair in both bacteria and human are :

1. Damage reversal
2. Base Excision repair (BER)
3. Nucleotide Excision repair (NER)
4. Mismatch repair (MMR)

5.1 Damage reversal

In this simplest form of DNA repair mechanism, a single-polypeptide-chain with the enzymatic properties binds to the damaged DNA part and restores the damage in a one-step reaction. Polypeptides playing role in this reaction are: 1. DNA prototype: removes cyclobutane pyrimidine dimers from DNA in a light-dependent process denominated photoreactivation (Carell et al., 2001). 2. O6-methylguanine-DNA methyltransferase I and II (DNA-alkyltransferase): removes modified bases O6-methylguanine, O4-alkylthymine and backbone-modified phosphate alkylphosphotriesters from DNA (Pegg, 2000). DNA-alkyltransferase is widespread as the activity of this group of the enzyme has been identified in extracts of Aspergillus nidulans, Saccharomyces cerevisiae, *Drosophila melanogaster*, fish and mammalian cells (Friedberg et al., 1995).

5.2 Base excision repair (BER)

DNA glycosylases initiate the BER. This enzyme catalyzes the hydrolysis of N-glycosylic bonds that link particular types of chemically modified bases to the deoxyribose-phosphate backbone (Olsen et al., 1989). The damaged part of the DNA is excised as a free base, and because of the loss of this base, an AP site is formed. AP endonucleases use AP sites as their target. These enzymes create incisions in DNA duplex by hydrolysis of phosphodiester bond immediately at 5’ or 3’ to each AP site (Kroken et al., 1997). Specific exonucleases called deoxyribophosphodieasterases (dRpase) remove Sugar-phosphate backbone from DNA. At the last step, DNA ligases and polymerases carry out the incorporation of a specific deoxyribonucleotide into the site of repair making correct pairing as shown in Fig.3. The BER mechanism is a cascade of enzymatic reactions in which each enzyme works separately and specifically in co-ordination with each other. The number of genes involved in the BER pathways is highly conserved from bacteria to humans (Soerensen et al., 2009) This pathway is maintained throughout the evolution. The presence of the AP endonucleases is shown in the bacteria, lower and higher eukaryotes such as *Trypanosoma cruzi* (Perez et al., 1999), (Perez et al., 1999), yeast, fish, plants, *C. elegans*, Drosophila and mammals (Friedberg et al., 1995). BER is proved to be effective DNA damage repair mechanism against DNA lesions such as a. Uracil b. Hydroxymethyluracil c. methylcytosine d. hypoxanthine e. G-T mispairs, f. 3-methyladenine g. 5,6-

hydrated thymine h. pyrimidine dimers (Friedberg et al., 1995). Specific DNA endonuclease identifies the specific lesion.

5.3 Nucleotide excision repair (NER)

A considerable amount of DNA helix gets distorted due to bulky base adducts in DNA formed by some endogenous or exogenous mutagens. The most widely studied and discussed DNA damaging agent is UV radiation which is capable of creating thymine dimers. Formation of the thymine dimers creates an approx. 300 bend in the DNA (Husain et al., 1998). Some unusual cross-liking occurs due to chemical agents that cause unwanted structural deformations and conformational changes which can be particularly hazardous as shown in figure 4. Such structures serve as target substrate for DNA endonucleases that create an incision in DNA. A big stretch of DNA consisting of several nucleotides to each side of damaged DNA is excised. Helicases catalyze the excision of the damaged fragment. Vacant DNA part is refilled by polymerases and finally sealed by ligases. In eukaryotic cells, NER is more complex pathway compare to bacteria. Different gene products are required in this multiple steps mechanism. NER in bacteria involves three proteins: UvrA, B, and C.

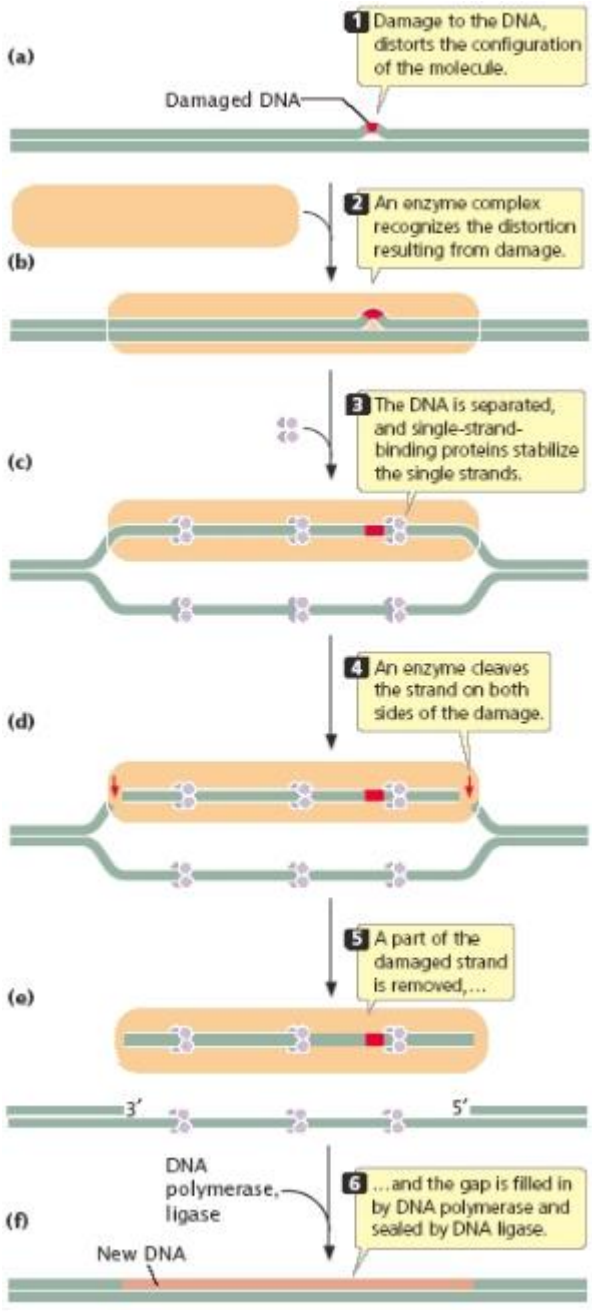

Fig. 4: Nucleotide excision repair mechanism (http://mb207.blogspot.nl/2012/01/part-ii-basic-genetic-mechanisms-dna_25.html)

5.4 Mismatch repair (MMR)

The DNA polymerase proofreading machinery can cause the error in its function as after replication, some frameshift and substitution mismatches may escape from the proofreading machinery. Mismatch repair system correct these errors by removing these substitution and frameshift mismatches. This increases DNA replication fidelity by 100 to 1000 fold.(Modrich and Lahue, 1989). In *E.coli*, proteins involved in MMR are encoded by MutS, MutL and MutH genes (Lahue et al., 1989). MutS protein homodimers recognize and bind to base-base mispairing and also insertion/deletion loop-outs (IDL). MutS in co-ordination with MulL protein homodimers, activate MutH protein to create an excision. This generates a nick in the

unmethylated i.e., newly synthesized strand. Exonucleases excise nicked strand (having IDL) and DNA polymerases and ligase resynthesize the DNA stretch that was excised (Marra and Schar, 1999).

As per current findings, the MMR mechanism in eukaryotic cells resembles that of E.coli at larger extent. At present, six MutS homolog and five MutL homologs are recognized that are called as MSH, MLH respectively. MSH2, MSH3, and MSH6 work as MMR machinery in the nucleus. MSH2-MSH6 heterodimers identify and replace the bases that are mismatched and loop out two bases. MSH2-MSH3 is also a heterodimer that is capable of identifying different sizes (Drumond et al., 1995, Marsischky et al., 1996). MSH4 and MSH5 together form a heterodimer that is involved in meiotic crossing-over and chromosome segregation (Nakagawa et al., 1999) as described in the Fig.5. MSH1 is targeted to the mitochondria and found as the necessary element for mitochondrial stability in yeast (Reenan and Kolodner, 1992).

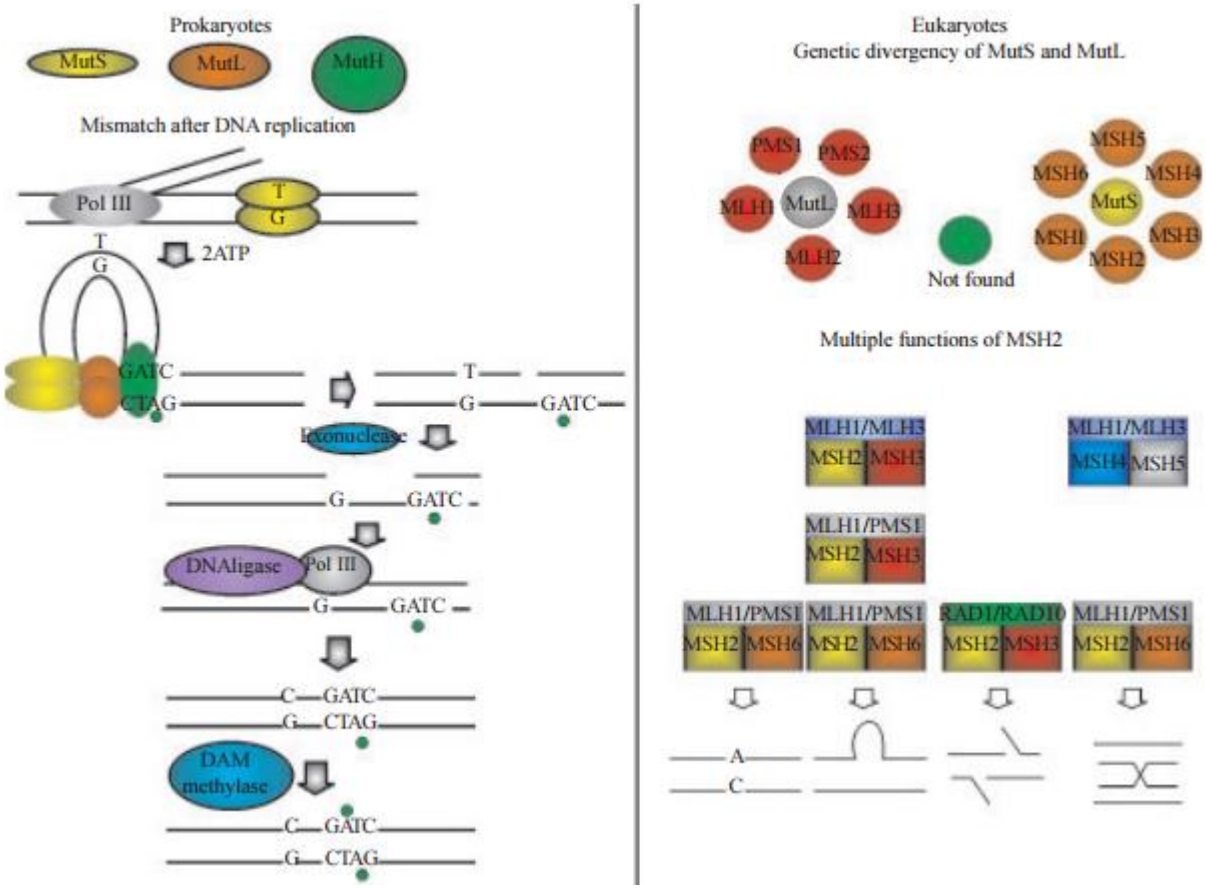

Fig. 5: (Left) Schematic representation of MMR mechanism in prokaryotes. The MutS homodimer protein binds to the DNA using the energy of the hydrolysis of two ATP molecules. The MutL homodimer protein then associated with the bottom of this loop and activates the endonuclease MutH. The MutH protein only nicks the unmethylated strand which contains the incorrect base. Afterward, the cleaved strand is submitted to exonuclease activity, DNA resynthesis, and ligation. (Right): schematic representation of the MMR mechanism in eukaryotic cells [paper III: Pinto L., et al., 2003]. The MutS proteins diverged into six orthologous genes, while MutL diverged into five other genes; these are denominated MSH and MLH, respectively. The MutH protein is not found in eukaryotes. The MSH and MLH proteins interact as a functional heterocomplex and repair several types of the substrates, such as mismatches, single-strand loops generated during microsatellite replication, DNA double-strand breaks, and holiday junctions from meiotic crossing- over (Pinto L., et al., 2003).

6. Model organisms used so far to study DNA damage repair systems

For the cancer research, human cell lines proved to be a great resource (de Megalhaes 2004, Hushizume et al., 2015); but there is the limitation to this model as human cell lines restrict relevance of the findings to the cellular aspects of the individual aging (Kim et al., 2016). They also don't contribute substantially to understand in vivo mechanisms involved in DNA damage repair and cancers. This led to the urgent need of some alternate strategy(s) that either share or mimic cancer processes in humans. Experimental animal models serve this key purpose in the investigation of the DNA damage repair systems and their translation into humans for cancer treatments and prevention.

To safeguard organisms against lethal effects of DNA damage, well-organized repair strategies each concentrating on different types of DNA damage are evolutionarily conserved from bacteria to human (Ishikawa et al., 2004). Protection of the integrity of the genome is one of the most important factors for the cancer prevention. In vivo evidence for this phenomenon is not available in sufficient amounts because of insufficient appropriate animal models (Ishikawa et al., 2004, Grosse et al., 2014). Sequences of the protein domains involved in the DNA repair were analyzed in details in two most studied model organisms, E.coli and S. cerevisiae and compared with the sequenced with the genomes of bacteria, archaea, and eukaryotes (Aravind et al., 1999). In this study, some uncharacterized and conserved domains of the proteins were identified that were not known earlier. These include four families of nucleases and a family of eukaryotic repair proteins. Some of the previously known conserved domains were also detected such as a modified helix –hairpin-helix nucleic acid binding domain in archaeal and eukaryotic RecA homologs (Aravind et al., 1999).

Invertebrates like flies and worms are well studied for tumors (Kaeberlein and Kennedy 2011, Kapahi et al., 2010, Kenyon 2010). An abnormal growth of the body tissues, led to cancers when they are malignant (Hanahan and Weinberg, 2011). In cnidarians and sponges reports of abnormal growth have been registered (Squires, 1965, Robert 2010, Kim et al., 2016). Successful model organisms used in cancer and DNA damage repair research include yeast, worms and flies (non-vertebrates) and zebrafish and mice (vertebrates). The spectrum of the aging of these different model organisms in captivity has the wide range from several weeks to years that mimic different features of the human cancers and DNA damage repair systems (Table 5).

Transgenically induced neoplasia in Hydractinia is an interesting example for the tumor studies (Millane R., et al., 2011). When disturbances occur in the differentiation of the interstitial stem cells (ISCs) into female gametes, a formation of the transplantable tumors into the two species of hydra: Hydra oligolectic and Pelmatohydra robusta have been observed (Domazet et al., 2014).

Recently, A naturally short-lived vertebrate: the African turquoise killifish *(Nothobranchius fuzeri*) has been proposed as the vertebrate model for cancer and aging research (Valdesalici and Cellerion, 2003). Some exceptionally long-lived species such as naked mole rat (up to 30 years), Brandt's rat (up to 40 years) and the bowhead whale (up to 200 years) also have been employed to perform comparative genomics studies to identify genes and residues that are uniquely changed or under positive selection in these organisms (Keane et al., 2015, Kim et al., 2011, Seim et al., 2013).

Table 5: Model systems to study human cancer and aging (adapted from: Kim et al., 2016)

Model Organism	Typical Lifespan duration	Number of mutants to date
Yeast	5-14 days	825
Worm	12-18 days at 20^0C	741
Fly	30-40 days	140
Zebrafish	36-42 months	19
Killifish	9-26 weeks	6
Mouse	2-3 years	112

6.1 Invertebrate models

S. cerevisiae or the budding yeast is a unicellular eukaryote which is used widely in cancer research (Henderson and Gottschling 2008, Herker et al., 2004, Sinclair and Guarente 1997, Verduyckt et al., 2016). Throughout the life span, budding yeast undergoes a limited number of replication events. Yeast replicative life span (RLS) is defined by the number of daughter cells produced by the mother cell. The survival time of the populations of non-dividing yeast cells is the measure of the chronological life span (CLS) (Herker et al., 2004, Kaeberlein, 2010, Longo et al., 1996). RLS and Survival time of the populations of non-dividing yeast cells are measures of the yeast survival.

Reduced food intake capacity (without malnutrition) which is known as Dietary restriction (DR) (Colman et al., 2014, Piper et al., 2011) is one of the better studied contributing factor capable of delaying aging and increase lifespan in several species however these models should be further investigated for effective DNA damage repair systems they can offer (Fontana and partridge, 2015). DR has shown to increase yeast RLS and CLS also. One of the most striking cellular effectors contributing to increasing life span is the target of rapamycin (TOR) molecular pathway (Bonawitz et al., 2007, Baeberlein et al., 2005, Powers et al., 2006). This is the molecular pathway involved in the protein synthesis and degradation in response to the nutrient quality and quantity (Stanfel et al., 2009) and it is largely conserved from yeast to mammals. There might be the relation between aging and impairment of this pathway as age-related disorders like diabetes and obesity are observed to have metabolic dysfunctioning in humans as a result of this impaired TOR pathway. Provided the fact that basic eukaryotic intracellular organelles and machinery in the yeast in conserved, it has been used as a model to study intracellular effects of the mutated genes playing role in the several cancers that are results of mis-regulated DNA damage repair pathwayds (Cooper et al., 2006, Khurana and Lindquist, 2010).

Since 1974, Caenorhabditis elegans (*C. elegans*), transparent soil nematode is being used as an experimental model system (Bernner, 1974). As it is multicellular organism and its life cycle can be entirely recapitulated in the Petri dish, *C. elegans* is a very useful model system. This worm is about 1mm in length in lab conditions in the adult form and lives only for some weeks. It's life cycle is been investigated in details ((http://www. wormatlas.org/). Many aging phenotypes of the worm are shared with other organisms including humans, for instance, decreased overall body motility, food consumption, a considerable increase in DNA damage, decreased metabolite rate, accumulation of age pigments and dramatic changes in age-dependent gene expression (Collins et al., 2008). During stages of the development, *C. elegans* can enter a stress-resistance biological state known as dauer

that is characterized by typical morphological changes and capacity to survive through starvation, temperature changes and other stressors (Gottlieb and Ruvkun, 1994, Lithgow et al., 1995,Riddle et al., 1981). Genes contributing to the regulation of the dauer formation in *C. elegans* also contribute to regulation of worm longevity and their function in regulating stress responses in conserved across many organisms, including humans (Kenyon et al., 1993). The worm is highly acquiescent to genetic manipulations (Klass, 1983) which have several allowed several screens that have brought some pathways in the attention that are involved in cancer conditions occurred due to mis-regulated DNA damage repair systems. Most importantly, these genetic screens provided the first evidence that a single gene can modulate longevity in the multicellular eukaryote (Kimura et al., 1997, Klass 1983, Lee et al., 2001, Ogg et al., 1997, Tissenbaun and Ruvkun, 1998). These results gave the strong foundation for the investigation of shared cellular organismal mechanisms like the stress responses and nutrient sensing that control longevity in multiple organisms and these findings are likely to be relevant to the human cancers (Kenyon, 2010, Kim, 2007, Lopez-Otin et al., 2013).

Having many key attributes the fruit fly, Drosophila melanogaster (D. melanogaster) is a well-established and valuable model organism. Some of the attributes are it's ease to maintain and that it is acquiescent to the manipulations using advanced genetic tools along with the availability of public resources including mutant and gene libraries (http://flybase.org/) (Matthews et al., 2005; Millburn et al., 2016). Wild-type D, melanogaster is an outstanding experimental model to study biology and cancer and effects of different interventions on overall life expectancy as it can survive just for few months in captivity. Even though modern flies and worms are phylogenetically equally related to the vertebrates (Mosaro and Austad, 2006), flies are having some more features that make them more closely similar to the higher vertebrates for instance, complex and centralised brain, a heart (Chintapalli et al., 2007), presence of multipotent adult stem cells in the midgut and the gonads (Micchelli and Perrimin, 2006; Ohlstein and Spradling, 2006; Wallenfang et al., 2006). Several fly models have been developed as this fly exhibits functional proximity to the vertebrates are useful for study of mammalian cancers specifically, the conditions like DNA damage accumulations in muscle, brain, cardiac and intestinal tissues (Demontis and Perrimon, 2010; Guo et al., 2014, Haddadi et al., 2014; Ocorr et al., 2007)

In the flatworms the only proliferating cells are the stem cells and can be eliminated by irradiation with no damage to differentiated cells. Effects of the fractionated irradiation schemes were investigated on survival, gene expression, morphology and regeneration in the *Macrostomum lignano.* Proliferating cells were most undetectable during first week post-treatment. Cell proliferation and gene expression were restored within 1 month in a dose-dependent manner following exposure to up to 150 Gy irradiation. During recovery, stem cells did not cross the midline but were restricted within lateral compartments. An accumulated dose of 210 Gy resulted in a lethal phenotype. Finding of this research demonstrate that *M. lignano* represents a suitable model system for elucidating the effect of irradiation on the stem cell system in the flatworms and for improving our understanding of the recovery potential of several damaged stem-cell systems (Mulder K., 2010) and to discover the novel DNA damage repair pathways.

6.2 Vertebrate Models

Zebrafish *(Danio rario)* has emerged as extreme;y successful vertebrate experimental model organism, and at present, it is widely used. Embryos of the Zebrafish are transparent and hence are

particularly acquiescent to live imaging studies in the early stages of development. These organisms have lower maintenance costs compared to mice, they produce many embryos and are amenable to large-scale genetic and pharmacological interventions. Based on these advantages as model organisms, zebrafish has provided fundamental knowledge of the molecular mechanisms underlying vertebrate development (Duboc et al., 2015; Kikuchi, 2015; Veldman and Lin, 2008). One of the most important attributes of the zebrafish is its regeneration capacity that makes it the suitable model for cancer research (Anchelin et al., 2011, Gilbert et al., 2014, Kishi et al., 2003, Van Houcke et al., 2015). Age-dependent mitochondrial dysfunction, telomere deterioration and protein oxidation are hallmarks of high accumulation of DNA damages due to insufficient or defective DNA damage repair systems (Kishi et al., 2003).

One of the most widely adapted model system to study the biology of mammalian cancers is the laboratory mouse, Mus musculus. Provided fact that basic physiological mechanism is highly conserved between mouse and humans, lab mouse as a model has helped to reveal many of the casual molecular mechanisms associated with DNA damage repair pathways. (Liao and Kennedy, 2014; Vanhooren and Libert, 2013). The life span of the available inbred mouse strains varies from 2 to 4 years (Yuan et al., 2011), that is very short and hence enable to test the models in relatively short time.

A considerable amount of the knowledge has emerged both clinically and experimentally that led to the fact that the DNA damage repair genes are tumour suppressors. Clinical consequences of the inherited defects of the DNA repair systems are deleterious and are apparent from several human cancer predisposition syndromes, for instance, NER comprised xeroderma pigmentosum (XP) and p53 deficient Li-Fraumeni syndrome (Ishiwaka et al., 2004). Because of the lack of the effective experimental models, experimental studies are affected and are insufficient to support the clinical evidence. From the individual study, in vivo experimental data has suggested the protective functions of the DNA repair machinery against chemical carcinogenesis. Three repair genes are selected for this study: O6-methylguanine-DNA methyltransferase gene (MGMT), XP group A gene (XPA) and p53.

Transgenic mouse line ‘ada’, overexpressing *E.coli* MGMT gene, was generated. This study of nitrosamine-induced hepatocarcinogenesis opened the fact that tumorigenesis was dramatically suppressed in this mouse line. MGMT is a rapid error-free DNA repair enzyme that eliminates alkylating lesions of O6-methylguanine (Sedgwick B., et al., 2002, Margison G., et al.,2003). O6-methylguanine is the most potent mutagenic lesion that preferentially pairs with the thyme creating G-C to A-T transition mutation (Sedgwick B., et al., 2002; Margison G., et al.,2003). This type DNA damage is repaired by direct reversal process catalyzed by MGMT. MGMT transfers methyl group of the O6-methylguanine moieties of the double-stranded DNA to cysteine residues of the MGMT molecule itself (Lindahl and Woord 1999; Friedberg 2003; Hoeijmakers 2001; Friedberg 2001; Mitchell 2003; Ishiwaka 2001; Sedgwick B., et al., 2002; Margison G., et al.,2003) . After treatment with the methylnitrosourea (MNU), in ada and control mice lines, it was confirmed that elevated levels of the MGMT in thymus efficiently protected mice from lymphomas (Dumenco 1993). The most striking differences between ada and control mice were observed in female given 5 mg od dimethylnitrosamine (DMNA) and sacrificed at 9 months. ada mice showed respectively less number of tumor multiplicity compare to control mice (13% vs. 68%) and no carcinoma was produced in ada mice. Similar results were obtained after about 11 months in males given 1 mg of DMNA but

carcinomas were induced by4% in ada mice (Fig 6). As can be observed from the figure, tumor-bearing livers are significantly fewer in ada mice.

XPA-deficient mouse line with selective impairment in the NER that is the animal model for human XPA showed the development of the skin tumors at the high incidence when exposed to 7,12-dimethylbenz[a]anthracene (DMBA)(Fig. 7), as they did when exposed to UV-B irradiation. Experimental methods targeting to the liver, lung, and tongue of XPA-deficient mouse were employed to addressed the intriguing problem of whether internal organs of XP patients were also prone to cancer. Patients with the XP are hypersensitive to UV and have a 1000 fold increased risk of skin cancer (Cleaver, 2002).

The importance of NER has been well established in the prevention of the UV-induced skin cancers by several experiments using XP-deficient mice. Literature survey also suggests that XP patients have

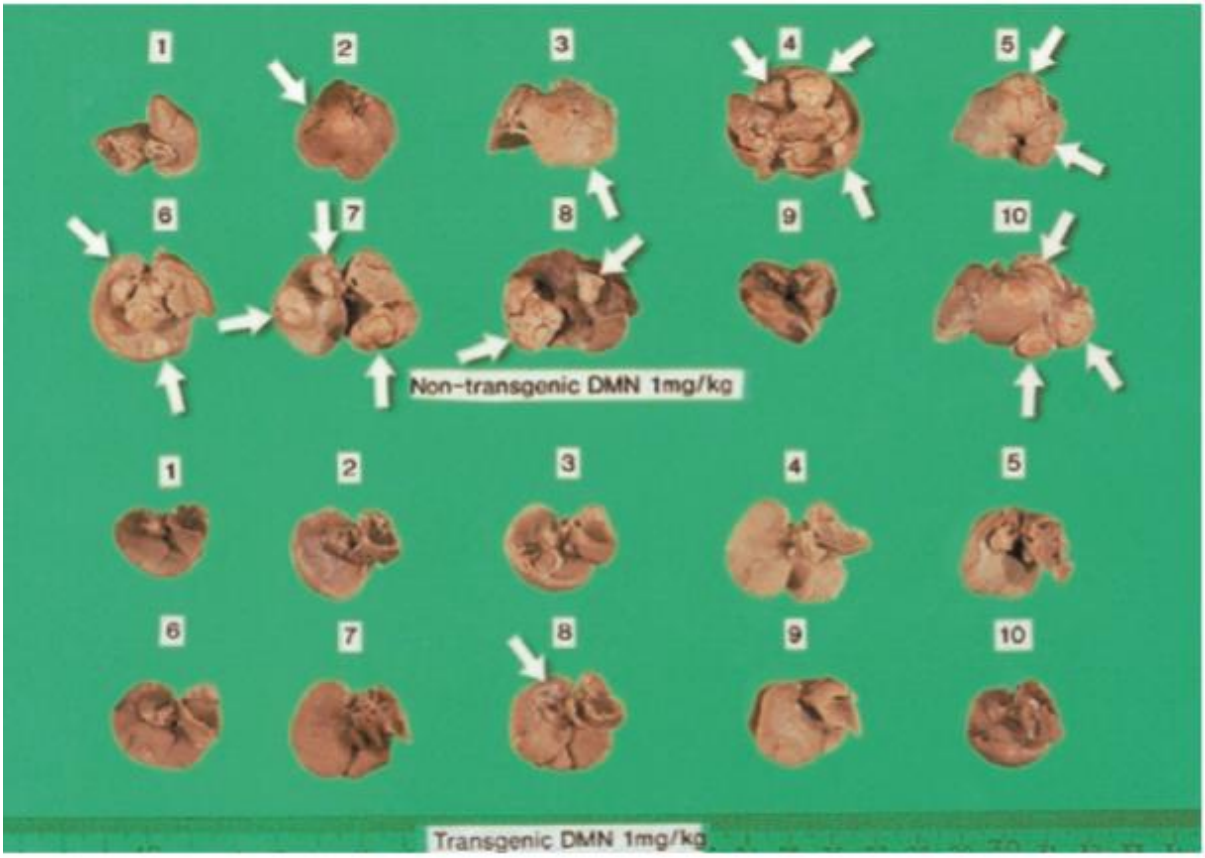

Fig. 6 Representation of the DMNA-induced liver tumors in male ada mice (transgenic) and control mice (non-transgenic). Arrows indicate tumors. (Ishiwaka et al., 2004).

10 -20 fold higher risk of cancers in internal organs (Hoeijmakers 2001; de Boer 1999; Kraemer 1984; Kraemer 1987; Santoh 1988, Kraemer 1994; de Boer 2000). At the end of this study (Ishiwaka et al., 2004) investigation was carried to test the possibility that, p53 might have direct significance to the early events in the brain tumorigenesis. This was achieved by transplacental exposure of the p53 deficient mice to ethylnitrosourea. p53 is located on the chromosome 17 p13 and it observed to be key tumor suppressor gene. Tumour suppressor function of this gene is also supported by the facts

that the gene is mutated in about half of the types of the cancers arising from different types of the tissues (Evan 2001; Guimaraes 2002). p53 is proved to be a gatekeeper for cancer prevention. It was concluded that DNA repair genes including MGMT, XPA and p53 actually protect against cancer.

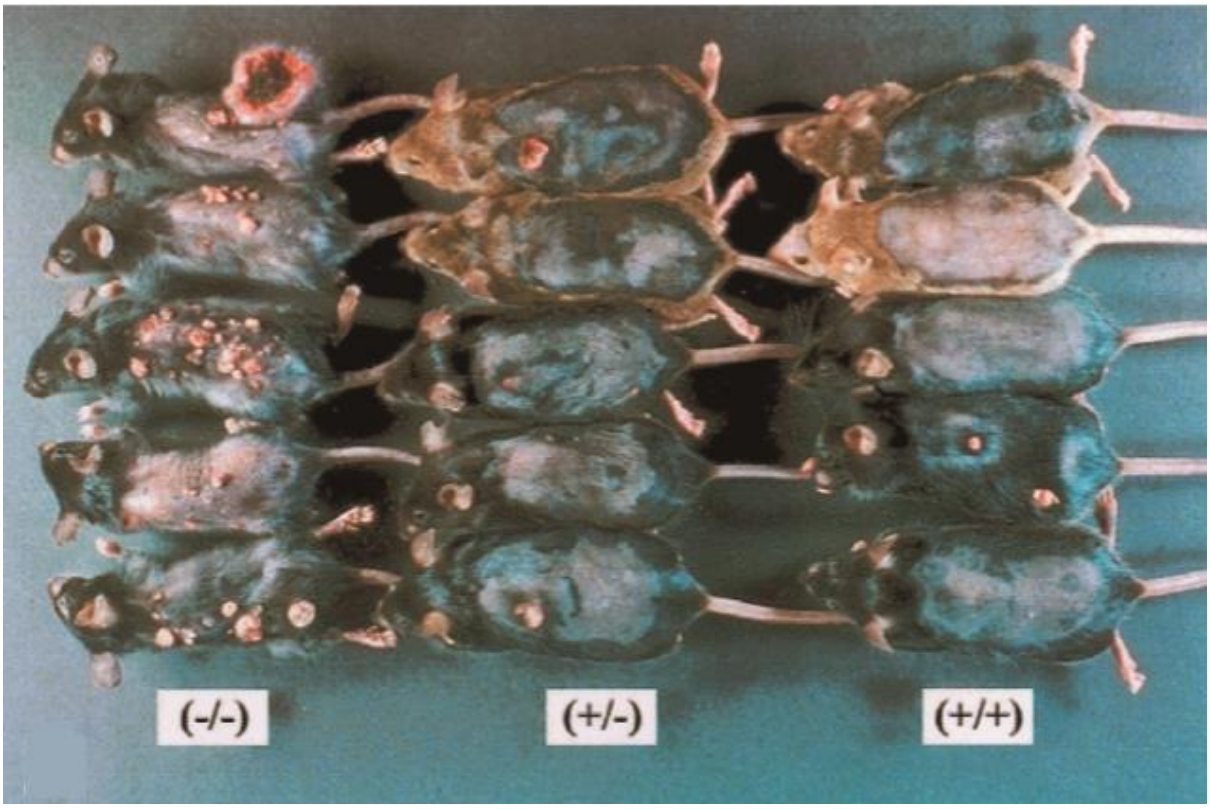

Fig.7 DMBA induced skin tumors in XPA –deficient (left), heterozygous (middle), and wild type (right) mice. As can be seen in the figure, tumors are more frequent in XPA-deficient mice.

It is proposed that dog can be the good model for the studies of the tumors. As DNA damage response pathways are key players during the progression of the tumors and in their treatment, these pathways are investigated in the canine. Canines can be potential future models to study DNA damage responses in vivo as wells as to translate human and veterinary medicine (Grosse et al., 2014). Dogs are proposed to be ideal model in many aspects of the cancer research. When canine DDR and human DDR pathways were compared it can look forward to the cancer research and treatment. Most important fact is that cancer occurring in the dogs and humans arise naturally with the age hence it can be compared. Histological appearances, tumor genetics, molecular targets, biological behavior and responses towards the therapies are some factors that humans and dogs share in common. Antibody cross-reactivities of the humans and canine proteins showed that DDR of dog is similar to the human cells to the greater extent (Grosse et al., 2014)

The newly emerging model African turquoise killifish (Nothobranchius fuzeri) (a teleost fish) with a natural life span ranging between 4 to 9 months has potential to be a promising model in aging research (Genade et al., 2005; Terzibasi et al., 2008). At present, turquoise killifish laboratory strains include the inbred 'GRZ' strain derived from an original population collected in 1968 (Genade et al., 2005; Valdesalici and Cellerino, 2003) and recently, several wild strains that were derived (Bartakova et al.,2013;Reichwaldetal.,2009;Terzibasietal.,2008). Most unique feature of the turquoise killifish is

it's life cycle. They are adapted to reach sexual maturity and reproduce during a very short period (Cellerino et al., 2015). Once hatched fish grows rapidly, as they are in need to complete sexual maturation and reproduce before the water evaporates (Podrabsky, 1999) (Fig. 8)

The turquoise killifish shows many molecular, cellular and physiological ageing phenotypes that are common with many other organisms including humans despite of its relatively short life span (Genade et al., 2005; Hartmann et al., 2009, 2011; Terzibasi et al., 2009, 2007; Valenzano et al., 2006b). Male turquoise killifish that are more colorful than females lose their body and tail color as well as their distinct patterning as they age (Fig. 8) similar to aging mammals progressively lose their hair and skin pigment with the age (Geyfman and Andersen, 2010). Short-lived vertebrate with old age also shows abnormal spine curvature, defective vision, and fin structure deterioration, decreased spontaneous locomotion activity, learning impairment (Genade et al., 2005; Valenzano et al., 2006b) and, most strikingly, an increased risk of cancer (Baumgart et al., 2015). Such macroscopic phenotypes recapitulate several complex age-dependent changes that occur in other vertebrates including mouse and humans (Vanhooren and Libert, 2013). An important feature of the killifish compared to other fishes is its rapid onset within 3-4 months of age. Age-dependent telomere attrition has linked to organismal aging in humans and in many other model organisms (Benetos et al., 2001; Harley et al., 1990; Lopez-Otin et al., 2013). Most striking feature of all shown by killifish is that killifish telomeres that are over four times shorter than in mice (Zhu et al., 1998), are comparable in length to human telomeres (Hartmann et al., 2009).

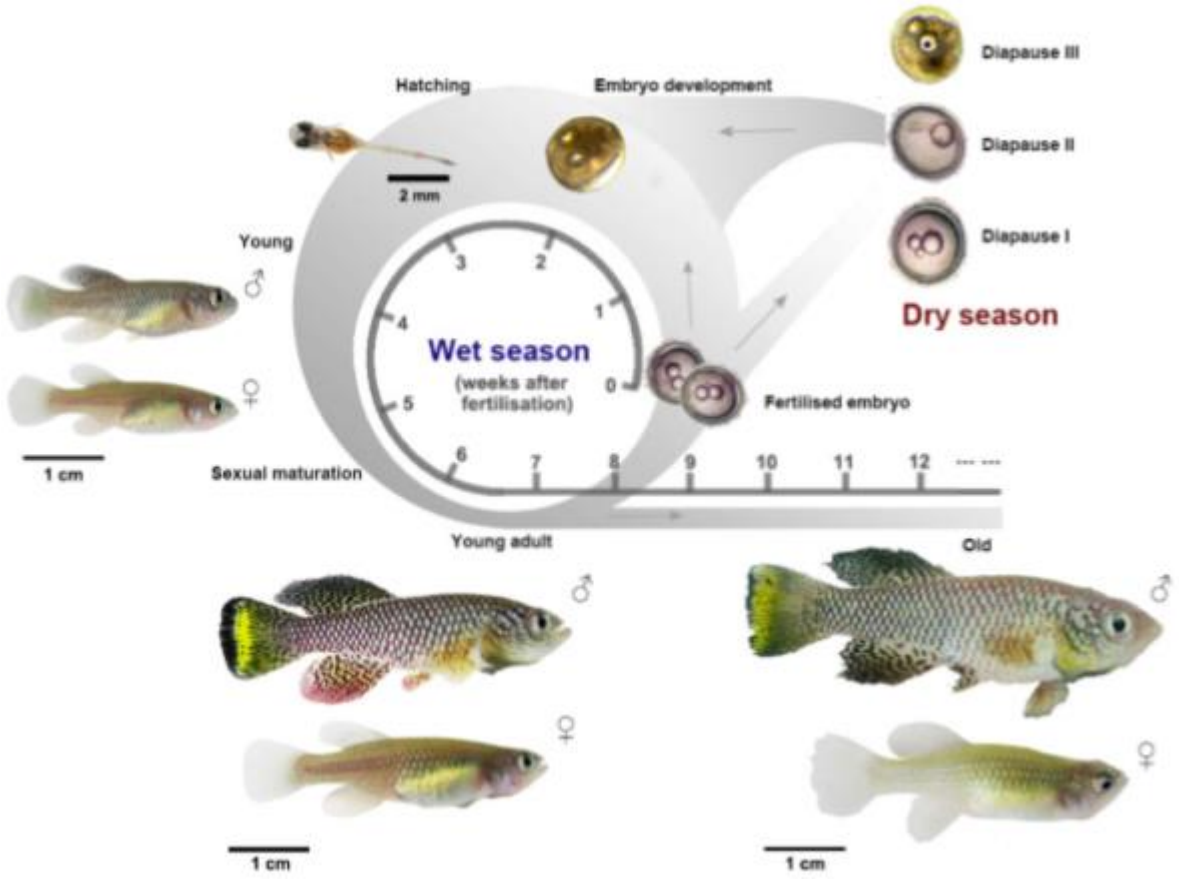

Fig. 8: Turquoise killifish life cycle (the time scale is based on the short-lived laboratory strain).

Embryos can enter normal development or a developmentally arrested state called diapause, which lasts from a few weeks to several months and protects killifish during the dry season in the wild. Diapause consists of three different stages called diapause I, II and III. During the wet season in the wild – and in laboratory conditions – hatched fry fully develop within 3-4 weeks and start spawning. Male fish are larger than females and have colorful fins and body, whereas the female fish are dull. Upon aging ('old'), fish lose body color, fin structure deteriorates and the spine becomes bent. The age for young, young adult and old fish is indicated in weeks. (Kim et al., 2016).

Killifish is the promising and efficient model for the study of vertebrate aging and cancer research as it uniquely combines short life span and life cycle with features specific to vertebrates and those are currently not available in currently used non-vertebrate model organisms. Specifically, it undergoes continues adult cellular proliferation and has adult stem cells in many tissues and adaptive immune system. It also shows a spontaneous age-dependent increased a risk of the cancer progression. Killifish has shortest life span among all vertebrate models in the captivity shares several age-associated phenotypes with other vertebrates including humans along with the diseases (Table 6). The killifish captive strains are highly capable of producing an abundance of offspring, facilitating transgenic line generation and genetic screenings. Given the fact that mice and zebrafish have comparatively long life span it is not practically feasible to functionally analyze such gene variants, particularly when most likely outcome if lifespan extension. Killifish has potential to fill the need for a short-lived vertebrate enabling variants linked to extreme longevity to be tested in a rapid testing in vertebrates of the genetic variants identified in worms and flies (de Magalhaes and Costa, 2009; Tacutu et al., 2013) making possible the translation of the findings from short-lived invertebrates to vertebrates. Recent genomic and transcriptomics data analysis in the turquoise killifish have revealed many orthologous genes to humans and other model organisms (Harel et al., 2015; Petzold et al., 2013; Reichwald et al., 2009) and hence detailed research should be conducted in order to investigate if there are any potential DNA damage repair pathways present in the killifish that can be translated to the humans.

Table 6: Turquoise killifish as a platform to test gene variants associated with human age-related dysfunctions (Table adapted from Kim et al., 2016)

Dysfunctions in human	Diseases examples	Key genes in human	Killifish orthologues
Genome instability	Cancer	P53, PTEN, PI3K, HER2, VEGFR, PARP	PTEN, PARP
	Aplastic anaemia	TERT, TERC, TRF1/2	TERT
	Dyskeratosis congenital	TERT, TERC, DKC	TERT, DKC1
	Werner syndrome	WRN	WRN
	Progeria	LMNA	LMNA
Mitochomdrial dysfunction	Alpers-Huttenlocher syndrome	POLG	POLG
	Ataxia neuropathy syndromes	POLG, C10orf2	POLG
	Leigh syndrome	MT-ATP6, SURF1	ATP6
	Neuropathy, ataxia, and retinitis pigmentosa	MT-ATP6	ATP6

Neurodegeneration	Alzheimer's disease Huntington's disease Parkinson's disease	APP, PSEN1/2, APOE, TREM2 HTT LRRK2, PINK1, SNCA, FBXO7, PARK2, PARK7/DJ-1,PLA2G6,VPS35,ATP13A2, DNAJC6, SYNJ1	APP,PSEN1/2 HTT LRRK2, PINK1, SCNA, FOXO7, PARK2, VPS35, PARK7, PLA2G6, ATP13A2, DNAJC6, SYNJ1

Metabolic dysfunction	Phenylketonuria Propionic academia Glycogen storage diseases Tay-Sachs disease Type 1 diabetes	PAH PCCA, PCCB G6PC, SLC37A4, GYS1/2, PYGL, PYGM HEXA HLA-DQA1, HLA-DQB1, HLA-DRB1	PAH PCCA, PCCB G6PC, SLC37A4, GYS1/2, PYGL, PYGM HEXA -
Autoimmune defects	Systemic lupus erythematosus Rheumatoid arthritis Multiple sclerosis	erythematosus HLA-A/B/C, HLA-DP/DQ/DR HLA-DRB1 HLA-DRB1, IL-7R	HLA-DPA1, HLA-DPB1, HLA-DQB2 - IL7R
Cardiovascular dysfunction	Wolff-Parkinson-White syndrome Progressive familial heart block McKusick-Kaufman syndrome	PRKAG2 SCN5A, TRPM4 MKKS	PRKAG2A/B SCN5A MKKS

7. Discussion and Conclusion

The rate of accumulation of DNA damage in the cells depend on the efficiency of their respective repair mechanism and also individual component damaged (Hart et al., 1974). When low efficacy DNA damage repair systems fail to remove DNA damages from DNA, DNA damages keep accumulating. Accumulation of the DNA damages increase rapidly during lifespan and that cause pathological conditions which might be fatal. Hence it can be predicted that there is the relation between lifespan of the species and its ability to get rid of DNA damage. DNA damage due to aging was studied in mice using DNA from such tissues to act as primers for in vitro nucleotide incorporation catalyzed by calf thymus polymerase. At the end of this study, it was found that old tissue acted as better primers than young ones and DNA from old tissues were having a large number of strand breaks compare to young ones and hence it can be predicted that DNA damage repair systems loose the efficiency with the ageing that lead to the accumulation of the DNA damage and ultimately progress into cancers.

Provide the fact that cancer mechanisms have been accepted as universal to all living beings, animal models are serving as an outstanding tool for the research. The biggest advantage of animal models is that they have the relatively short life span and the introduction of genetic modification is possible. DNA repair pathways can be studied in unicellular and multicellular eukaryotes like *D. melanogaster, C. elegans* (Oliveria et al., 2010, Soerensen M., et al., 2009; Scheckhuber C and Osiewacz H., 2008). Results have shown that different eukaryotic models have notably contributed to the knowledge of the DNA repair mechanisms in aging process in humans.

Table 4: Estimate number of changes in *E.coli* and mammalian cells DNA induced by lethal dose of UV radiation (Table adapted from Kim et al., 2016)

	D_{37}	Single breaks	Double breaks	Dimers
E.coli				
Log phase				
Wild type	5-6 krad	25-30	~1	
polA$_1$	1	5		
recA	1	5		
Stationary phase				
Wild type	18	80	~4	
uvrA	15	75	~4	
recA	2	10	~1	
wild type	500 ergs/mm^2			3000
Mammalian Cells	200 rad 100 ergs/mm^2 (254 nm)	1000	15	4 X 10^6

D_{37}: dose that leaves 37% of the initial number surviving

As can be seen from the table, mammalian cells and *E.coli* have difference of three to four orders of magnitude more dimers than double stranded breaks that are induced by comparable dose of ioninzing radiatons. If at D_{37}, number of perticular type of alteration is same then in is interesting to see if there are any alterantion that might be lethal for instance, pyrimidine dimer in *E.coli* uvrA recA. Formation of one dimer is not lethal for wild type cells but aggregated effects of the 3000 dimers may cause lethality. Such high number of dimers may stop repair system from work pertucularly when repair pathway invloved step by step enzymatic reactions.

Principle radiation induced changes in DNA

1. **Double-strand breaks**: Lethal in extracellular phage, in *E.coli* but not in *M. radiodurans* or mouse lyphoma cells
2. **Single-strand break**: lethal in single starnded phage but not in mammalian cells (with effective and unsaturated repair system)and wild-type bacteria.
3. **Base damage after ioninzing radiation exposure**: Lethal in some phages, excised from *M. radiodurans* DNA and might be lethal in saturated repair system or repair-deficient cells.
4. **Interstrand links**: Lethal in uvrA revA cells repaired in uv-resistant bacteria and mammalian cells.
5. **Pyrimidine Dimers:** Not generted in baceterial spores in suspension. These are lethal in phage, bacteria. In wild type bacteria and mammalian cells dimers can make repair system saturate.
6. **UV induced chain braks in BrdUrd-sustituted DNA:** formed by free radicals in DNA. Lethal to phage, bacterial cells but observed to be repaired by mammalian cells rapidly.

From research on human samples, it is clear that misregulated DNA damage repair (DDR) pathways are crucial in tumor development and treatment of the tumors as well. Compare to in-vertebrate models discussed here, the vertebrate models like mice are more attractive ones as they already show to own the efficient DNA damage repair systems however invertebrate models are potential models to understand the molecular mechanisms underlying the DNA damages and DNA damage repair systems. Killifish and dogs can be potential experimental animals to model the cancers caused by DNA damages. Experimental studies with such models can help to understand the DNA damage repair systems in more details and increase their efficiency, introduce novel modifications and ultimately translate them into humans for cancer treatments and prevention.

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