Greater Expression of DNA Repair Pathways in Sharks vs. Rays/Skates Based on Transcriptomic Analyses

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Abstract

Elasmobranchs are an understudied taxon of cartilaginous fishes. DNA repair studies have been done in very few elasmobranchs. Because DNA repair maintains the integrity of the genetic code, it is important for the survival of elasmobranchs in increasingly polluted oceans. Oil spills, for example, have been shown to cause DNA adducts in ocean creatures. We hypothesized that four elasmobranch species would show differential DNA repair expression. Dermal cells were harvested from nurse sharks (Ginglymostoma cirratum), spiny dogfish (also considered to be sharks) (Squalus acanthias), yellow stingrays (Urobatis jamaicensis), and little skates (Leucoraja erinacea) and RNA was isolated. RNA sequencing was performed using the holocephalan Australian ghostshark (Callorhincus milii) reference genome. RNA samples from the sharks manifested significantly higher expression than those of rays/skates in 4/5 major DNA repair pathways (Base Excision, Nucleotide Excision, Mismatch Repair, and Homologous Recombination). Each of the four pathways of DNA repair manifested differential expression of pathway-specific genes (xpa, gtf2h3, mpg, rad51c,d, mlh1, ercc3, ercc6, xrcc3, parp1, atr, brip1, top3A, blm, ddb1, fancd2, etc.). One gene, polD2, was consistently elevated in the sharks vs. rays/skates in all four pathways. Although we studied RNA from skin, polD2, a subunit of polD, has been proposed to contribute to the spreading and amplification of hypermutations in sharks by generating higher diversity of the T cell receptor repertoire. In summary, with increased expression of four major DNA repair pathways, sharks may be more successful than rays/skates in surviving the genotoxic effects of increasingly polluted oceans.

Introduction

Maintaining DNA integrity is critical for preventing diseases such as cancer. Assessing DNA repair and the potential for genetic damage is essential for identifying environmental threats to ocean life and informing conservation efforts. Pharmaceuticals, agrochemicals, and environmental shifts due to climate change all present threats to marine life that involve DNA damage and repair. Although saltwater studies have previously assessed crustaceans, bivalves and bony fishes (Clade *Osteichthyes*), fewer DNA damage and repair related studies have been done on cartilaginous fishes (Clade *Chondrichthyes*), which includes Subclass Holocephali (chimaeras) and Subclass Elasmobranchii (sharks, rays, skates) (**Fig. 1**) (Alves, 2022; Consales and Marsili, 2021, Marques, 2025).

All the major DNA repair pathways have been found in bony fishes within Infraclass Teleostei (Kienzler et al., 2013). Additionally, many teleosts have an additional repair pathway for UV damage known as photoenzymatic repair (*per*), which is absent in placental mammals. None of these DNA repair pathways have previously been demonstrated in elasmobranchs, illustrating the novelty of this study.

Elasmobranch fishes have ecological relevance because of their relatively long life spans and wide dissemination, with an additional role as a food source to humans. Ecologically, elasmobranchs generally occupy high trophic roles in many marine ecosystems and exert top-down pressures in local food webs. As predators, elasmobranchs also have associated vulnerability to the bioaccumulation of chemical contaminants.

Because of overfishing and environmental contamination, over a third of elasmobranch species are threatened (Dulvy et al., 2021), especially those with coastal, sedentary lifestyles or those that are directly in contact with the oceanic sediment. Chemical contamination assessments of elasmobranchs involve accumulation of metals and organic pollutants known to cause antioxidant response, DNA alterations, and DNA damage (Alves, 2022; Chynel, 2021). Global climate change has caused variations in salinity and acidification, complications that can

contribute to DNA alterations (Alva, 2017; Alves, 2022). The research on DNA damage in ocean species has primarily addressed these risks in bony fishes (Wirgin and Walman, 1998; Simoniello et al., 2009; Canedo et al., 2021).

Little is known about the pollution tolerance of elasmobranchs. Reviews like Gelsleichter & Walker (2010) and Bezerra et al. (2019) focus mostly on contaminant levels rather than DNA repair mechanisms to remediate specific forms of DNA damage. Other reviews like Tiktak et al. (2020) focus on pollutants from a human consumption perspective. Marra et al. (2019) found molecular sequences associated with DNA repair mechanisms in white shark genomes but did not assess functionality or relative RNA expression. However, many elasmobranchs spend time in coastal, likely more polluted waters for at least reproductive purposes, such as nursery grounds for pups and egg cases (Heupel et al., 2018).

The present study provides novel information on DNA repair pathways for four coastal elasmobranchs.

Methods

Elasmobranch sampling

All the work in this study was covered by NSU IACUC protocol 2017-DK-01 to DWK. Nurse sharks (*Ginglymostoma cirratum*, n=3) and yellow stingrays (*Urobatis jamaicensis*, n=3) were hand captured near Fort Lauderdale, Florida, while spiny dogfish (*Squalus acanthias*, n=3) and little skates (*Leucoraja erinacea*, n=3) were captured by commercial fishing vessels near Chatham, Massachusetts. Specimens were brought onto the fishing vessel or dock, and then sterile techniques were performed on the live animal to remove a small sample of skin. The tissue sample was then placed into a sterile, RNAse-free Eppendorf tube and snap-frozen in a dry ice and ethanol mixture. Samples were subsequently stored at -80°C.

RNA Isolation and Sequencing

Tissue was pulse homogenized on ice for 20-30 seconds in the presence of RNAse inhibitor in a fume hood. RNA was isolated using the Qiagen RNAeasy Kit, and RNA integrity and concentration were determined using an Agilent TapeStation. RNA samples were then subjected to library preparation using Illumina's TrueSeq Stranded Total RNA library generation kit and sequenced on a 2x150 base-pairs paired-end run using the NextSeq 500 High Output (300-cycle; 400 million read flow cell). The data were delivered as Fastq files, which were analyzed using Partek Flow software (Partek Inc., MO).

Data Analysis

Alignment of exonic reads was performed using BWA-MEM (Burrows-Wheeler Aligner) in Partek Flow software using the Australian ghostshark (*Callorhincus milli*) reference genome. Aligned reads were then quantified using the Partek Annotation Model E/M to produce gene counts. Gene set ANOVA, an unsupervised data analysis, was used to determine pathway differences between the sharks vs. rays/skates. Since four major DNA repair pathways were among the top 50 significant pathways, we report those here. Gene expression data were collected, based on the genes within each pathway found in gene set ANOVA analysis. Finally, normalization was performed using counts per million reads.

RNA sequencing results were based on three biologic RNA samples from each species of elasmobranch. Four species in total were used (2 sharks and 2 rays/skates). Data were expressed as the mean ± standard error for each group. One-tailed Student's t-tests were performed using Microsoft Excel (version 16.63) and GraphPad Prism software (version 9.3.1). A p-value of < 0.10 was considered to indicate a statistically significant difference for both pathway and individual DNA repair gene expression.

Results

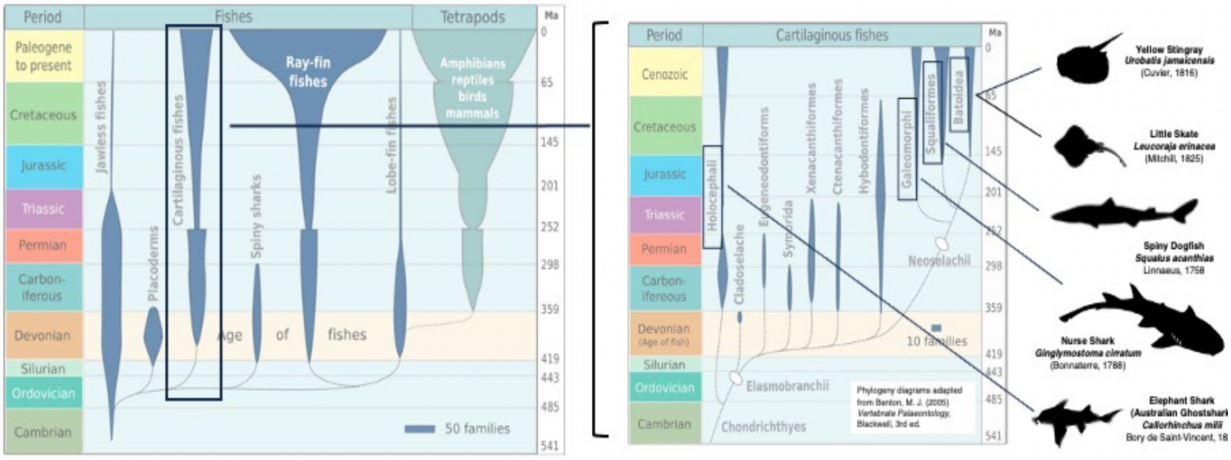

Fig. 1. Evolution of cartilaginous fishes from the Devonian to the present to show time scales of each phylogeny divergence. The width of the spindles in each sub-figure are proportional to the number of families as a rough estimate of diversity. On the right are visual representations of each of the cartilaginous Chondrichthyan fish species used in the analysis, corresponding to different phylogenetic groups. Fish images are public domain obtained through PhyloPic.org.

Chondrichthyan fishes (combined Holocephalan chimaeras and elasmobranch sharks, skates, and rays) diverged from bony fishes – including the sarcopterygian fishes (and Tetrapoda) – during the Ordovician Period (485-443 mybp) (**Fig. 1**). Batoids are one of the more recent groups to appear in Neoselachii, appearing at the end of the Jurassic Period (145 mybp). Note that these eras are only approximate, since most fossils of elasmobranchs are from teeth, dentine-covered placoid scales, and other hard structures (e.g., dorsal spines on spiny dogfish), which makes specimen-based, physical character phylogenies difficult. However, these broad phylogenetic differences between Galeomorphs and Squaliforms are consistent throughout several analyses (Maisey et al., 2004; Naylor et al., 2012), especially as distinct from Batoidea (Aschliman et al., 2012) and others have repeatedly confirmed the differences between Rajids (skates) and Myliobatiformes (rays).

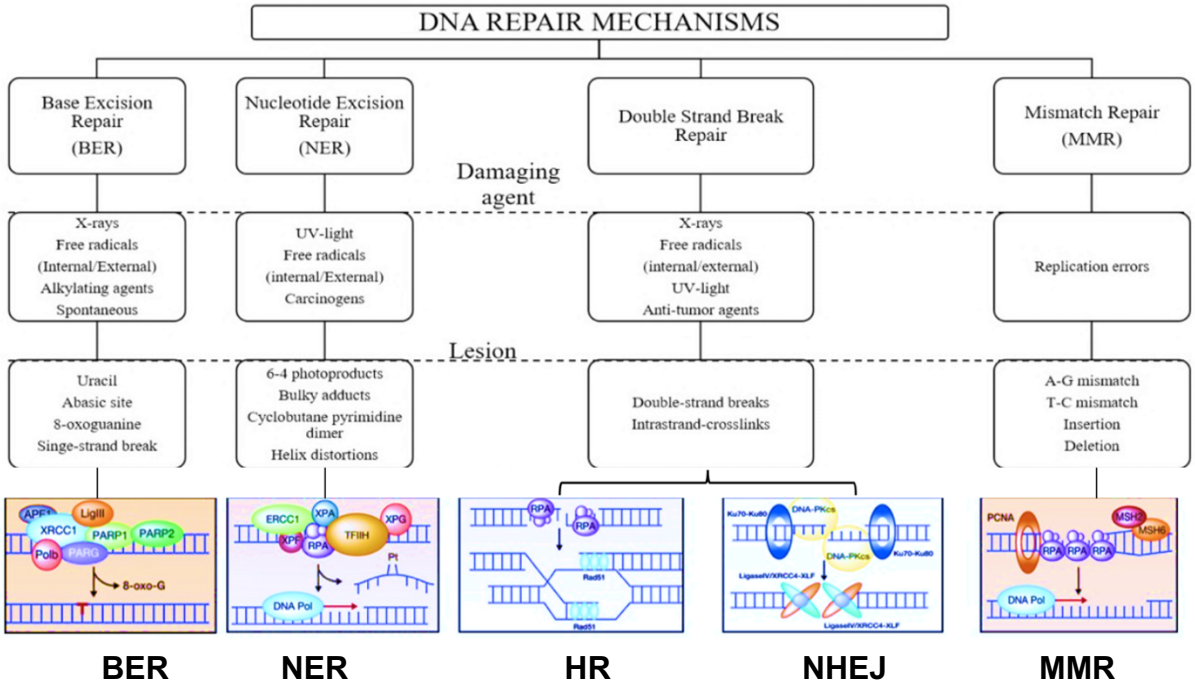

Fig. 2. DNA repair mechanisms are shown.

There are multiple major types of DNA damages (base damage, bulky adducts and single-stranded crosslinks, double stranded breaks (blunt-ended or sticky ended) and mismatched bases after replication. These damages are resolved by 5 distinct pathways of DNA repair (**Fig. 2**). Base Excision Repair (BER) and Nucleotide Excision Repair (NER) remediate abnormal geometry in the DNA helix generated from single strand damages. BER repairs only the base that is damaged using evolutionarily conserved and specific glycosylases. NER resolves damages involving alkylating agents (common as pollutants) and single-stranded crosslinks by removing the area around the damage and resynthesizing a long patch (27-30 bp). Double stranded helix damage is resolved either by homologous recombination (HR) or non-homologous end joining (NHEJ).

Four of the five major DNA repair pathways were found to be significantly upregulated in sharks vs. ray/skates (**Table 1**): BER (**Fig. 3**), NER (**Fig. 4**), HR (**Fig. 5**) and MMR (**Fig. 7**). The Fanconi Anemia pathway was also included (**Table 1, Fig. 6**) because it is a disease state that includes components of double stranded and single stranded DNA repair pathways. It also includes related pathways such as cell cycle. The only pathway not shown to be differentially regulated by sharks vs. rays/ skates was NHEJ. It is remarkable that 4/5 pathways of repair show the same trend (higher in sharks) and an analysis of the major genes involved was performed to determine if genes in common between the pathways were involved. The polymerase delta2 subunit of *polD* was common to multiple pathways (BER, NER, HR and MMR) and significantly higher in sharks vs. rays/ skates. Polymerase delta is indispensable for leading and lagging strand synthesis in replication. *polD2* does not encode the catalytic subunit. Another polymerase, polymerase epsilon subunits 2 and 3 was also expressed with sharks being significantly higher than rays/ skates in BER, and *polE3* in NER. *polE* is involved in leading strand synthesis in replication, genome stability and cell cycle control.

There were however pathway specific genes in each of the four pathways showing higher expression in sharks vs. ray/skates so the pathway statistical trends were not driven by a small number of overlapping genes like the polymerases.

Gene Set Description	Fold Change	P Value	FDR Step Up
Fanconi Anemia	1.78	3.13E-5	2.6E-4
Homologous Recombination	5.42	3.43E-7	6.24E-6
Base Excision Repair	8.88	1.41E-4	9.65E-4
Nucleotide Excision Repair	4.76	1.01E-6	1.38E-5
Mismatch Repair	11.08	4.35E-6	4.75E-5

Table 1. Differential DNA Repair Related Pathways, showing differential expression of the entire pathway for sharks vs. rays/skates. P values and FDR values were evaluated for these pathways as a whole. FDR step up is the most stringent criterion for analysis of RNA sequencing. NHEJ is the only major pathway of DNA repair that is not differentially expressed between these groups. Fanconi anemia is a disease-related pathway. It includes aspects of NER (nucleotide excision repair) and of HR (homologous recombination) a form of double-strand break repair.

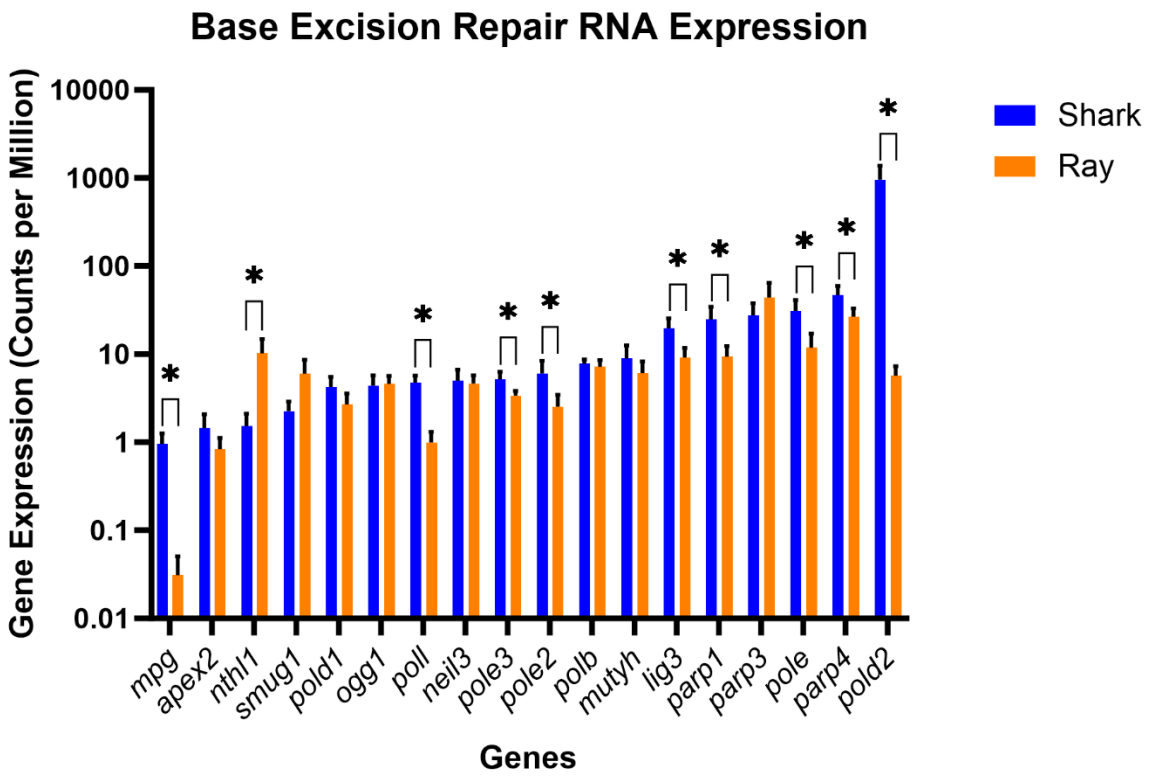

Fig. 3. RNA expression for genes involved in the **Base Excision Repair Pathway**. 9/18 genes were significantly higher in sharks vs. rays/skates: *mpg, polL, polE, polE2, polE3, lig3, parp1, parp4 and polD2*. *polD2* is the major polymerase involved in replication and repair. *nthl1* encodes a DNA glycosylase and is significantly higher in rays/skates vs. shark.

Nine genes were significantly higher in sharks vs. rays/skates: *mpg, polL, polE, polE2, polE3, lig3, parp1, parp4,* and *polD2*. *polD* is the major polymerase involved in replication and repair. N-methylpurine-DNA glycosylase (*mpg*) initiates BER by removing a large variety of deaminated, alkylated and lipid-peroxidation induced purine adducts. The *parp1* gene is involved in DNA repair and chromatin remodeling. It is active in single stranded, double stranded DNA repair pathways and BER. At least 11 distinct glycosylases are known in BER in mammals. Ligase 3 (*lig3*) closes the single-stranded nicks made by BER (Krokan and Bjoras, 2013).

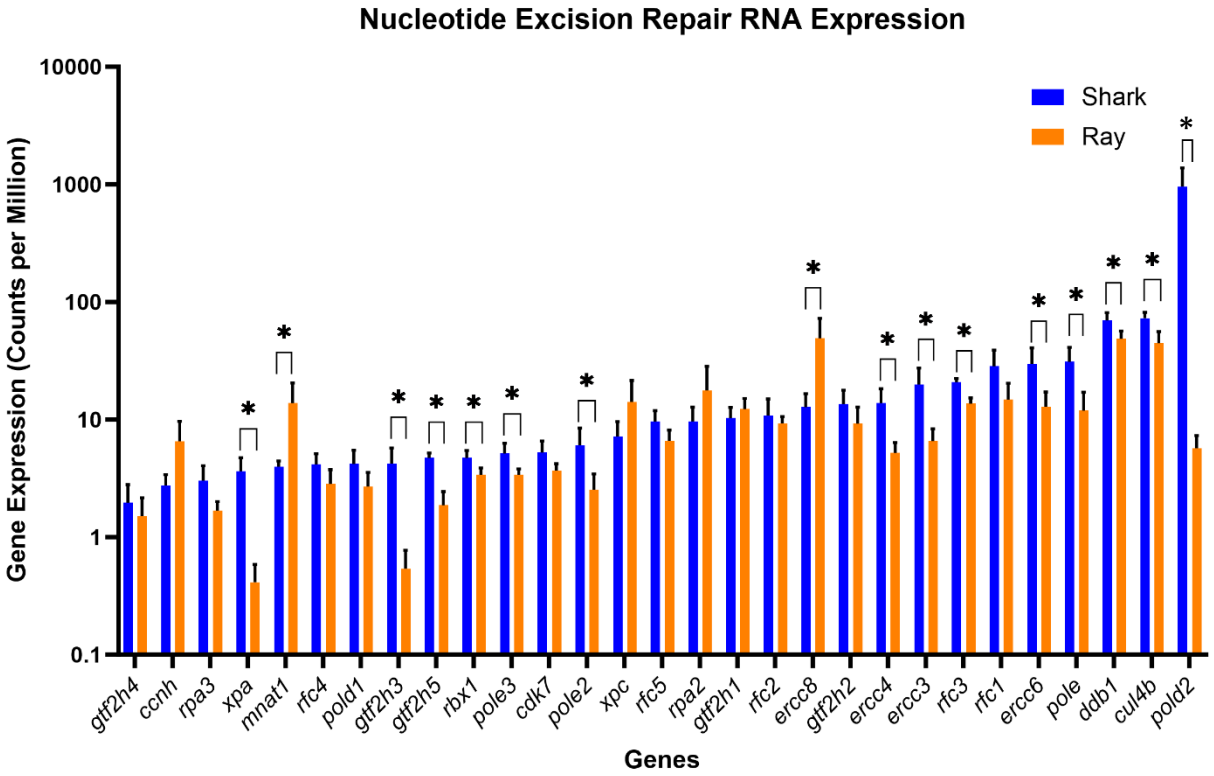

Fig. 4 Gene expression for genes involved in the **Nucleotide Excision Repair Pathway**. 14/29 genes were significantly higher in sharks vs rays/skates: *xpa, gtf2h3, gtf2h5, rbx1, polE3, polE2, ercc4, ercc3, rfc3, ercc6, polE, ddb1, cul4b,* and *polD2.* Several other genes show the same trend without significance. 2/29 gene is significantly higher in rays/skates compared with sharks: *mnat1* and *csa/ercc8* which is involved in transcription coupled NER.

NER is a pre-replicative DNA repair pathway which causes replication to pause so that the repairosome can repair the damage before the DNA is replicated avoiding mutation in the progeny strands. Fourteen genes were significantly higher in sharks vs. rays/skates: *xpa, gtf2h3, gtf2h5, rbx1, polE, polE2, polE3, ercc3, ercc4, ercc6, rfc3, ddb1, cul4b,* and *polD2.* Two genes were significantly higher in rays/skates compared with sharks: *mnat1* and *csa/ercc8* which is involved in transcription coupled NER. The *gtf2h* gene products are involved in DNA unwinding, recruitment of downstream DNA repair factors and verification of bulky lesions on DNA. These proteins are part of the transcription complexe as well as NER. *xpa* in concert with damaged DNA initiates repair by binding to damaged sites and stimulates the 5'-3' helicase activity of *xpd* and the DNA translocase activity of *xpb*. The product of the *ercc4/xpf* gene interacts with *ercc1* which plays a crucial role in DNA repair and recombination. The *ercc3* gene

product is involved in DNA repair and transcription and is a key component of the TFIIH complex (Krasikova et al., 2021).

The *csa* gene product is involved in transcription coupled NER as opposed to global genomic NER. This gene is expressed at higher RNA levels in rays/skates vs. sharks.

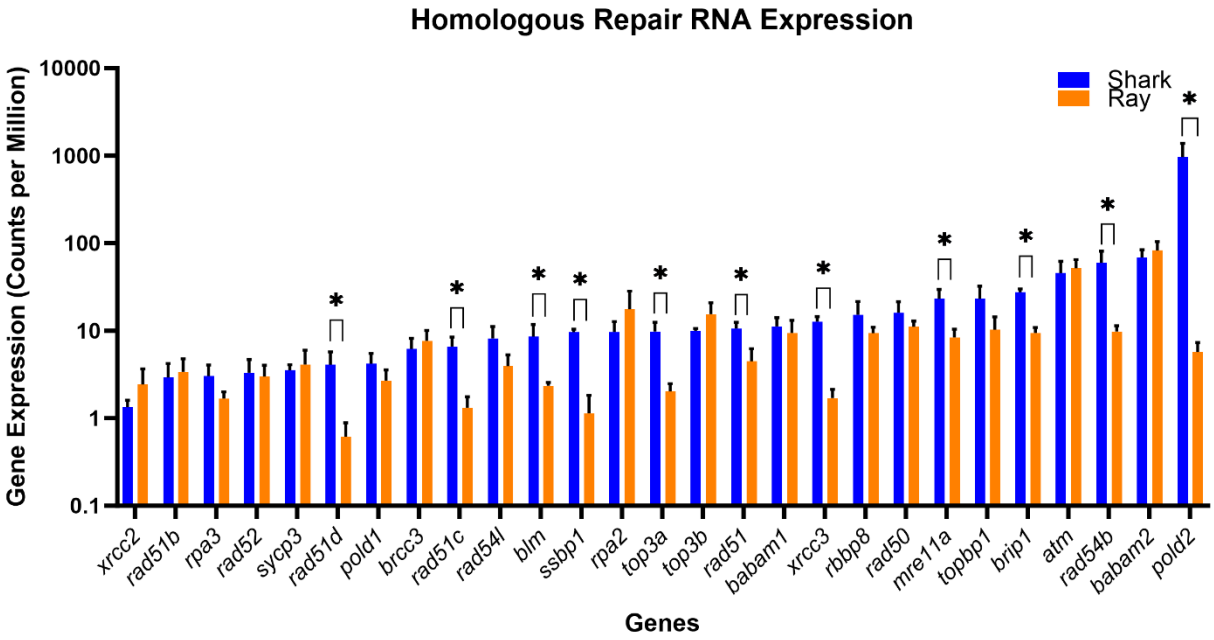

Fig. 5. RNA expression for genes involved in the **Homologous Recombination Repair Pathway**. 11/27 genes show significantly higher shark vs. ray/skate expression: *rad51D, rad51C, blm, ssbp1, top3a, rad51, xrcc3, mre11a, brip1, rad54b,* and *pold2*.

The pathway of DNA repair that did not show differential RNA expression between these groups was NHEJ. NHEJ exists in zebrafish so the finding that NHEJ is not differentially expressed in elasmobranchs cannot be explained by lack of homology. NHEJ and HR both repair double stranded breaks, however NHEJ is a faster, more efficient pathway directly ligating the broken ends of DNA, although often with some loss of information. NHEJ is inherently more mutagenic than HR (Stinson and Loparo, 2021).

In the HR pathway eleven genes show significantly greater expression in sharks vs. rays/skates (*rad51*, *rad51c*, *rad51d*, *blm*, *ssbp1*, *top3A*, *xrcc3*, *mre11a*, *brip1*, *rad54b*, *polD2*). The RNAs for *rad 51, rad51c*, *rad51d* show this difference. *rad51c* and *rad51d* are tumor suppressor genes in mammals with known mutations associated with breast and ovarian cancers. The *xrcc3* gene product, another oncogene, is part of the *rad51* paralog protein complex (Uhrig et al., 2024). The *top3A* gene product (topoisomerase 3 alpha) resolves Holliday junctions and is implicated in Bloom's syndrome-like disorder when mutated (Martin et al., 2018).

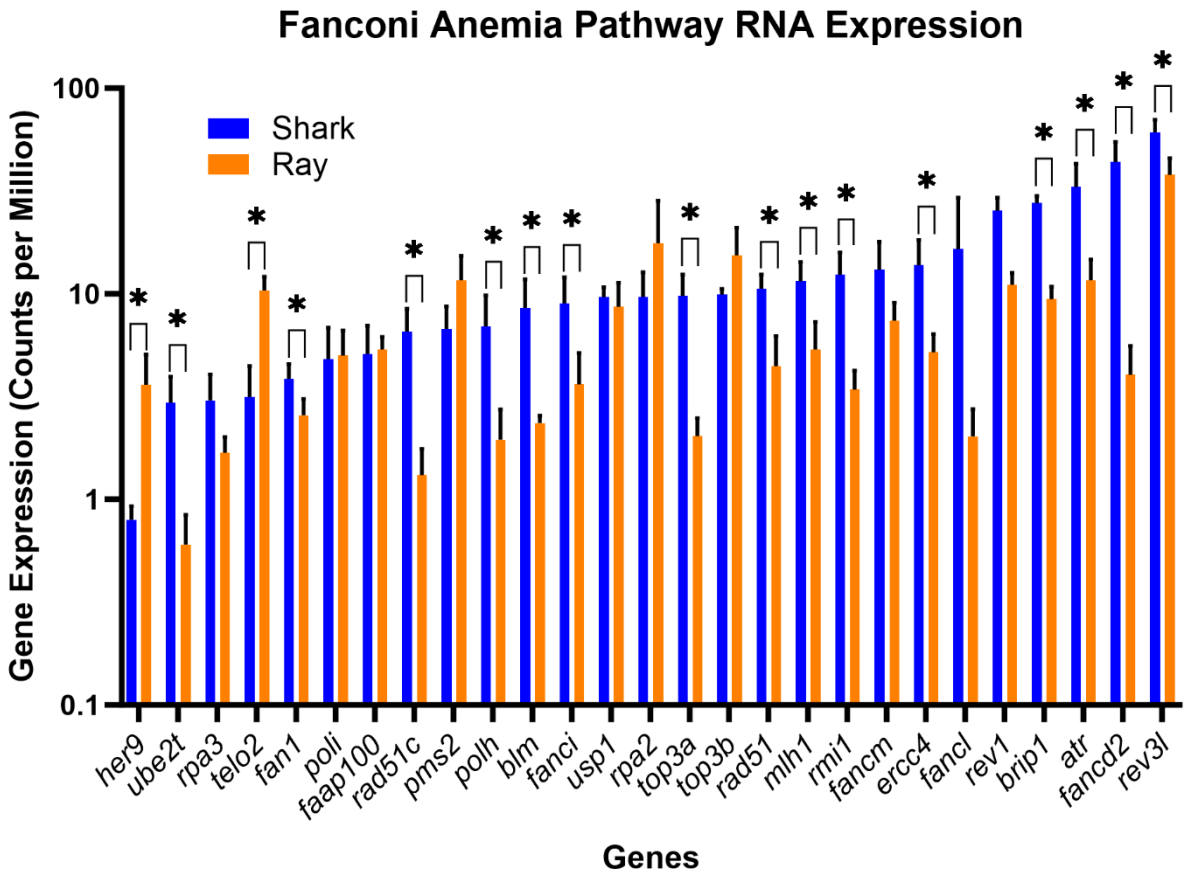

Fig. 6. RNA expression for genes involved in the **Fanconi Anemia Pathway.** 15/27 genes show significantly higher RNA expression in sharks vs. rays/skates: *ube2T, fan1, rad51C, polH, blm, fanci, top3A, rad51, mlh1, rmi1, ercc4, brip1, atr, fancd2, rev3L*. Two genes (*her9*, *telo2*) show the opposite pattern with sharks being lower than rays/skates.

The Fanconi Anemia (FA) pathway is included in this analysis although it is not a pathway of DNA repair but is instead a disease state, that includes aspects of double strand and single strand repair as well as replication (Moreno et al., 2021). In addition, the FA pathway also reveals functions that are related to other aspects of genomic instability like cell cycle regulation. The *atr* gene encodes a protein kinase, a monitor of DNA damage that is involved in cell cycle checkpoint regulation. The 15 genes expressed at higher levels in shark vs. ray/skate are: *ube2T*, *fan1*, *rad51C*, *polH*, *blm*, *fanci*, *top3A*, *rad51*, *mlh1*, *rmi1*, *ercc4*, *brip1*, *atr*, *fancd2*, *rev3L*. The gene *ercc4* is part of the NER pathway. The *mlh1* gene product is part of mismatch repair. The gene product of *top3A* (topoisomerase gene) relieves supercoiling upstream of replication forks and is included in the replication pathway. The *rad51* and *rad51c* genes are part of the HR pathway (Uhrig et al., 2024). The *brip1 gene* encodes a DNA helicase that interacts with the *brca1* gene (Wang et al., 2023).

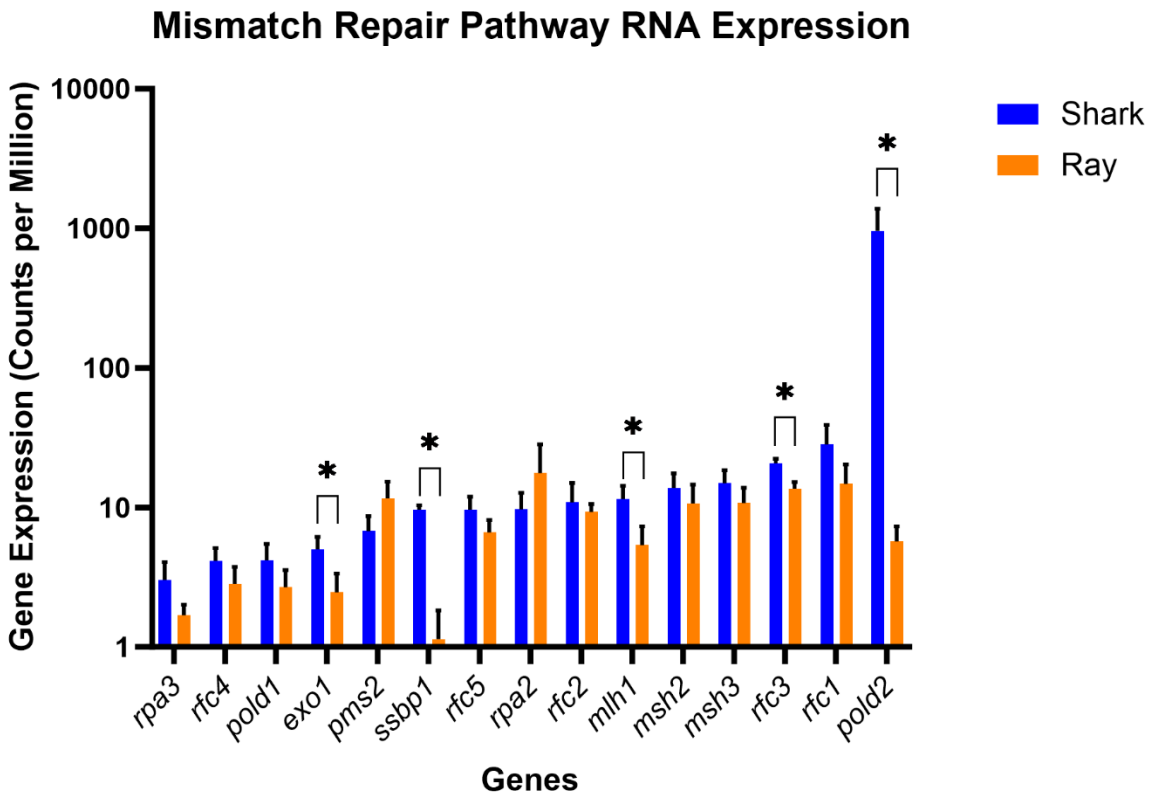

Fig. 7. RNA expression for genes involved in the **Mismatch Repair Pathway** which is a post-replicative repair pathway. 5/15 genes show significantly higher RNA expression in sharks vs. rays/ skates: *exo1, ssbp1, mlh1, rfc3,* and *polD2*.

In Mismatch Repair, five genes show significantly higher expression in sharks vs. rays/skates (*exo1*, *ssbp1*, *mlh1*, *rfc3*, *polD2*). The replication protein C factor (*rfc*) plays an important role in MMR, acting as a clamp loader, loading *pcna* onto DNA which is essential for the *mut* endonuclease activity on the mismatched nucleotide. *mlh1* is part of a protein complex that detects and binds to mismatches. Mutation in this gene is associated with colorectal cancer (Pećina-Šlaus et al., 2020).

Genes that overlap in two or more pathways include *polD2*, *polE*, *rfc, ercc4, Rad51, ssbp1*.

Discussion

Numerous freshwater game fishes have been studied for functional DNA repair (Keinzler, et al., 2013). However, similar DNA repair studies are presently lacking for many ocean species including elasmobranchs. Fewer still have involved examination of the transcriptome for DNA repair pathways as a whole, or looking at the expression of individual genes that are unique to these pathways. In addition to pathway analysis, our study has shown that *polD2* which is differentially expressed in all of these pathways at higher RNA levels in sharks vs. rays/skates.

Biologically, the four species studied occupy two different types of habitats: nurse shark and yellow stingray are both found in sub-tropical and shallow waters (1-25 m depths), while spiny dogfish and little skate are both temperate and in deeper waters (10-200 m). Given these habitat differences, nurse shark and yellow stingray are exposed to DNA-damaging UV

wavelengths which could be pertinent to NER gene expression and function, although this is not reflected in these results. Both the yellow stingray and little skate are mostly scale-less and subject to lacerations, whereas the spiny dogfish and (especially) the nurse shark have extremely strong skin and placoid scales, so the differences in DNA repair likely is not based on external integument.

Despite a close phylogenetic relationship between the four species of elasmobranchs, we see a clear distinction in gene expression patterns for 4/5 major pathways of DNA repair with the 2 sharks being significantly higher in gene expression. The related pathways of DNA replication and p53 signaling show the same trend (data not shown). Although DNA repair and replication are related, one does not drive the other, in other words, high replication does not necessarily mean high repair (Latimer et al., 1996; Latimer et al., 2003).

DNA repair genes are considered tumor suppressor genes. Many of the genes that show differential gene expression in this study with higher expression in sharks vs. rays/skates are mutated in human cancers (eg. *brip1, top3a, xpa, rad51, atr, mlh1, fanci, ercc4, rfc, polD, polE*, etc) (Mendiratta et al., 2021).

RNA expression is not equivalent to function, but in our previous work in cancer, we have found a strong correlation between the transcriptomic patterns and the functional patterns for NER (Latimer et al., 2010). DNA repair genes are expressed at low levels compared to many other classes of genes. This work suggests that sharks could be more successful in surviving polluted waters with genotoxic contaminants than rays/skates based on 4/5 significantly different pathways of DNA repair. One can envision these repair pathways being advantageous in the context of oil spills, for example. Carcinogens (specifically alkylating agents) are present in oil and petrochemicals (Rodrigues et al., 2010). Chemicals used to disperse oil, permeabilize cell membranes, creating greater nuclear exposure from these genotoxic carcinogens.

An alternative aspect of DNA repair involves somatic hypermutation which has been found in sharks in T cell specific genes *tcr* gamma and delta variable regions (Diaz et al., 1998; Zhu and Hsu, 2020). This hypermutation is believed to play a role in increasing the diversity of the T cell receptor repertoire, allowing sharks to respond to a wider variety of pathogens. This hypermutation is a function of certain DNA repair related genes such as error prone DNA polymerases (E, epsilon or D, delta) (Diaz et al., 1998; Zhu and Hu, 2010).

Although hypermutation is documented in sharks in cells of the immune system, this study used the skin of all four species of elasmobranch. Immune cells could have been present in the skin. NER is known to be tissue specific in mammals (Latimer et al.1996; Latimer et al., 2003; Latimer et al., 2008; Latimer et al., 2015) and remediates 2 types of UV based damages. HR is also known to be tissue specific in mammals, being more active in stem cells and actively proliferating cells (Tichy et al., 2010; Bishop and Schiestl, 2002). Tissue specific DNA repair has not yet been explored in elasmobranchs.

NER in particular has also been positively associated with longevity in mammals (Diderich et al., 2011), presumably because it minimizes mutation in the genetic code by remediating bulky adducts and intra-strand crosslinks using a long patch. Spiny dogfish (*ca.* 75 yr) and nurse shark (*ca.* 20 yr) are longer-lived than little skate (*ca.* 4 yr) or yellow ray (*ca.* 3 yr). Our data in fish are therefore consistent with the mammalian theory that lifespan is associated with the ability to repair DNA via the NER pathway (Diderich et al., 2011; Tian et al., 2017). However, for other pathways of repair, correlation with lifespan is understudied.

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