Aminoglycosides and Nonaminoglycosides Influence Readthrough of Premature Stop Codons In XPC Fibroblasts

Eric Bowman Jr[1,2,3], Laila Al-Eryani[1], Sikandar G. Khan[1], Kenneth Kraemer[1]

[1]DNA Repair Section, LCBG, NCI, Bethesda, MD, [2]College of Medicine, Howard University, Washington, D.C., [3]CRI SIPNCI, Bethesda, MD,

Introduction

- Xeroderma Pigmentosum (XP) is a genetically inherited recessive disorder
- XP increases risk of skin cancer[1]:
  - nonmelanoma→10,000-fold,
  - melanomas → 2,000-fold
  - tongue cancers →100,000-fold
- Estimated XP incidences in the USA are 1 in 1,000,000[1], 1 in 20,000 in Japan [5], and approximately 2.3/ million live births in Western Europe [5]
- XP has seven different complementation groups (A-G) translated for nucleotide excision DNA repair when exposed and damaged to UV light [1]
- Mutation(s) in the XP genes result in a much slower rate of DNA repair

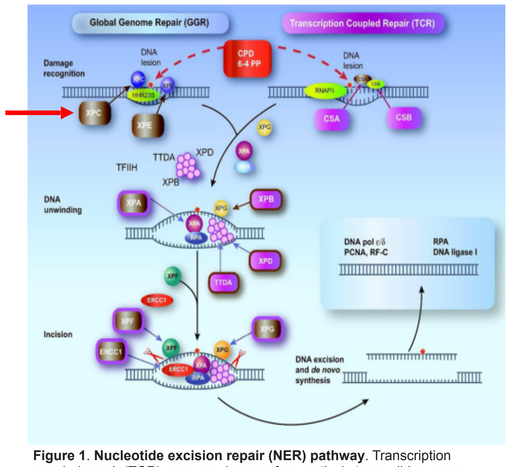


Figure 1. **Nucleotide excision repair (NER) pathway**. Transcription coupled repair (TCR) removes damage from actively transcribing genes while global genome repair (GGR) removes damage from the remainder of the genome. [1]

- Some XP patients become severely sunburned after minimal sun exposure with phenotypic expressions of lentigines and progressive neurological degeneration (XPA, XPD, XPG)
- XPC increases the likelihood of developing, early-onset freckles and skin cancer [1]
- Unlike the other complementation groups, XPC (and XPE) patients do not get burned on minimal sun exposure [1]
- XPC patients do not exhibit neurodegeneration [1]
- Premature stop codons (PTC's) cause the formation of an immature mRNA and thus, protein that is nonfunctional. Immature mRNA is degraded by nonsense-mediated decay (NMD)[3]
- About 12% of human genetic disorders, including XP complementation group C (XPC), involve mutations causing PTC's [3]
- PTC's in XPC gene cause NMD of the XPC-mRNA resulting in XPC phenotypes [3]

Readthrough of Premature Termination Stop Codons

Studies have shown that some aminoglycoside and nonaminoglycoside compounds can randomly substitute a nucleotide base in the premature termination stop codon sequence to a different amino acid leading to a "readthrough" of the RNA that produces functional protein [3]

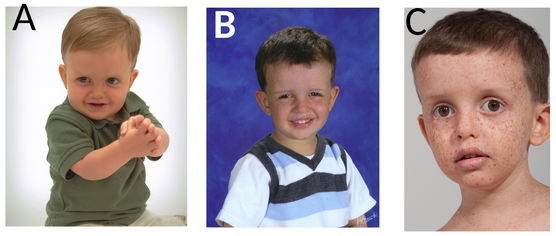
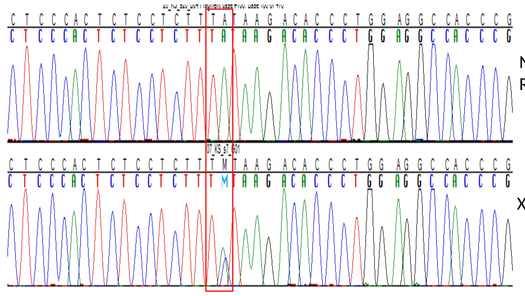


Figure 2. Patient XP449BE age progression A. 11 months old, B 3 years old, and C 4 years old displaying progression of freckling. He had SCC removed from right lower eyelid at age 3 yr.

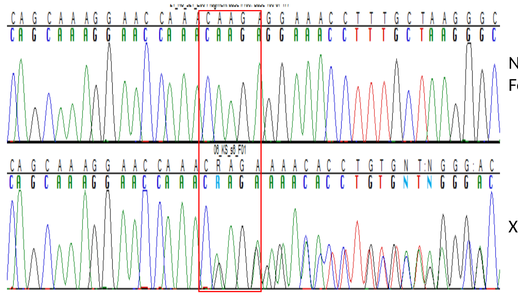
Objective

Determine the effect of aminoglycosides (geneticin, kanamycin) and nonaminoglycoside (amlexanox) on XPC mRNA levels in fibroblasts from XPC patient with PTC mutation at exon 9

Figure 3. Mutations in XP449BE XPC patient



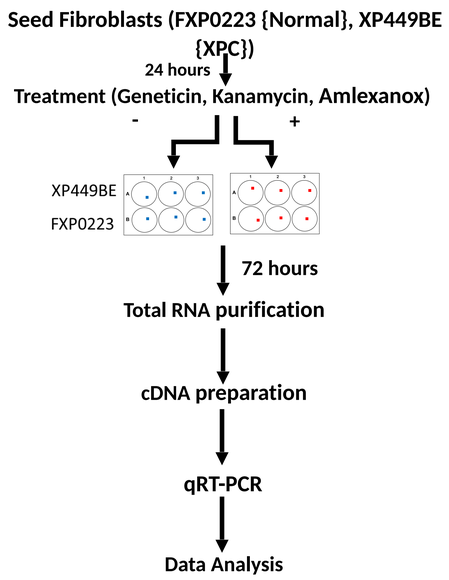
Mutation allele I: *XPC* Exon 9: c. 1290 A>C (T>G), Y430* (TAT (Y) >TAG (stop)) – **PRIMARY PREMATURE STOP CODON**



Mutation allele II: *XPC* Exon 9: c. 1396 –7 del. AA (fs. 431Stop 5 codons downstream) – **SECONDARY PREMATURE STOP CODON**

Materials and Methods

Figure 4.



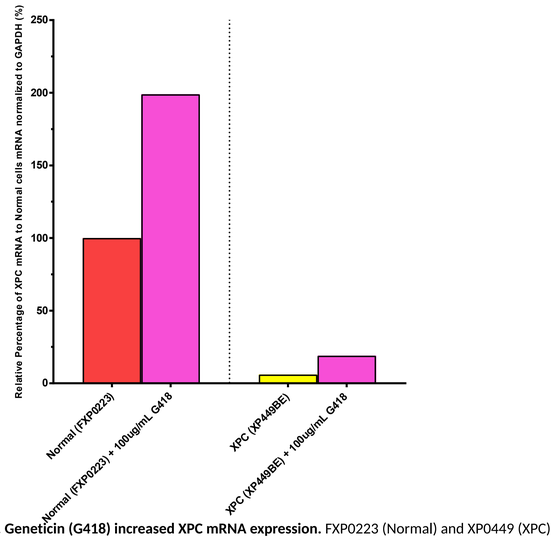


Figure 6. Geneticin (G418) increased XPC mRNA expression. FXP0223 (Normal) and XP0449 (XPC) fibroblasts were independently exposed to Geneticin (100 µg/mL) for 72 hours in DMEM media supplied with 10% FBS and 1% L-Glutamine at 37 C in (8% CO_2). Femtograms of XPC expression were normalized first to GAPDH femtograms then the normalized expression of each sample was normalized to the normal control sample (FXP0223) with no treatment.

Results

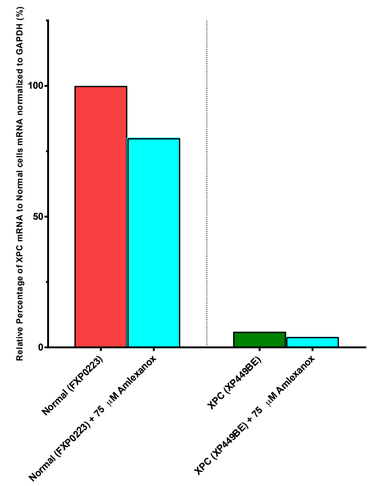


Figure 5. XPC mRNA expression decreased after treatment with 75 µM of amlexanox. FXP0223 and XP0449 fibroblasts were independently exposed to amlexanox for 72 hours in DMEM media supplied with 10% FBS and 1% L-Glutamine at 37 C in (8% CO_2). Femtograms of XPC expression were normalized first to GAPDH femtograms then the normalized expression of each sample was normalized to the normal control sample (FXP0223) with no treatment.

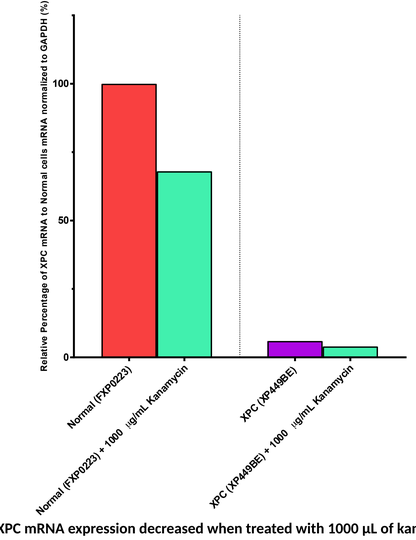


Figure 7. XPC mRNA expression decreased when treated with 1000 µL of kanamycin. FXP0223 and XP0449 fibroblasts were independently exposed to kanamycin for 72 hours in DMEM media supplied with 10% FBS and 1% L-Glutamine at 37 C in (8% CO_2). Femtograms of XPC expression were normalized first to GAPDH femtograms then the normalized expression of each sample was normalized to the normal control sample (FXP0223) with no treatment.

Conclusion

- Geneticin increased XPC mRNA expression in both normal and XPC fibroblasts
- The XPC mRNA expression decreased with both amlexanox and kanamycin treatments in normal and XPC fibroblasts

Future Directions

- Cell line specific toxicity studies should be continued to determine which concentrations with least toxicity are efficient to increase readthrough of mRNA

References

1 Digiovanna, J. J., & Kraemer, K. H. (2012). Shining a Light on Xeroderma Pigmentosum. *Journal of Investigative Dermatology*, *132*(3), 785-796. doi:10.1038/jid.2011.426

2 Kuschal, C., Khan, S. G., Enk, B., Digiovanna, J. J., & Kraemer, K. H. (2015). Readthrough of stop codons by use of aminoglycosides in cells from xeroderma pigmentosum group C patients. *Experimental Dermatology*, *24*(4), 296-297. doi:10.1111/exd.12655

3 Kuschal, C., Digiovanna, J. J., Khan, S. G., Gatti, R. A., & Kraemer, K. H. (2013). Repair of UV photolesions in xeroderma pigmentosum group C cells induced by translational readthrough of premature termination codons. *Proceedings of the National Academy of Sciences*, *110*(48), 19483-19488. doi:10.1073/pnas.1312088110

4 Baradaran-Heravi, A., Niesser, J., Balgi, A. D., Choi, K., Zimmerman, C., South, A. P., . . . Roberge, M. (2017). Gentamicin B1 is a minor gentamicin component with major nonsense mutation suppression activity. *Proceedings of the National Academy of Sciences*, *114*(13), 3479-3484. doi:10.1073/pnas.1620982114

5 Lehmann, Alan R, et al. "Xeroderma Pigmentosum." *Orphanet Journal of Rare Diseases*, vol. 6, no. 1, 1 Nov. 2011, p. 70., doi:10.1186/1750-1172-6-70.