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Evaluating Splice Variant Expression of the DNA Polymerase Beta Gene in BE2C and HEK Cells Exposed to *Borrelia burgdorferi*

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While *Borrelia burgdorferi*, the primary bacterial factor for Lyme Disease, is expected to cause significant DNA damage, it is not known how some repair pathways react under such circumstances. The aim of this experiment is to detect how infection with such bacteria affects the expression of the normal and exon 2-lacking (ΔE2) variants of the DNA Polymerase Beta (PolB) gene by means of quantitative PCR (qPCR). The method of probing for expression of splice variants that was used is a bit unorthodox because it must be certain that the exon is present or missing in the respective samples [1]. In this experiment, a probe was designed to overlap the junction between exons 1 and 2 to ensure expression in the samples intended to have exon 2 present if it was indeed present, and wouldn't if it was absent. Inversely, in the ΔE2 samples, the probe from exon 1 to exon 3 would only bind if exon 2 was absent, similarly ensuring the samples' contents. After HEK and BE2C cells were cultured, some were infected with *B. burgdorferi*, and both had their RNA extracted to synthesize cDNA which was used in the qPCR assay.

The expression of the PolB gene was shown to have a consistent upregulation when cells are exposed to *Borrelia burgdorferi*, as great as twelve-fold. This is regardless of whether it was the complete or ΔE2 isoform. However, it appears the bacteria affect BE2C cells as little as three-fold and as great as ten-fold higher than HEK, and the complete splice variant tends to be more upregulated than the ΔE2. It is novel that in every sample, there was a statistically significant upregulation. The fold change that had the smallest increase was of 1.8, which is still a relatively meaningful difference.

This study shows that infected cells have a significant upregulation of the PolB gene indicating an increased use of base-excision repair pathways. Whether this is a cellular response to the bacteria in an attempt to repair the damage being done to the DNA or a bacterial response of *Borrelia burgdorferi,* itself, is unknown. High expression of the PolB protein normally indicates a relatively large amount of damage to a cell's DNA [2]. Isoform ΔE2 causes a frame shift of the PolB gene. This could have a different effect than the full-length variant, as well as splice variants with other exon deletions. Previous studies have shown nucleotide-excision repair is a pathway often associated with Lyme disease, which makes base-excision repair seem much less investigated [3-5].

In the future, each of the qPCR product should be run on a gel and sequenced to determine different mutations and such that may have occurred during infection. Furthermore, other exon deletions such as ΔE4, ΔE9, ΔE10 or parallel deletions such as ΔE2/9 and ΔE2/10 should be investigated. If the PolB gene expression is impaired due to the *Borrelia burgdorferi* infection, then an in-depth analysis may provide some insight on the overall repair process and its contribution to pathogenesis of Lyme diseases.

References

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