The 62nd Science and Engineering Fair of Houston

Immunotherapy of Human Prostate Cancer Utilizing the Bacterial Protein Flagellin

Kevin Song
Michael E. DeBakey High School for Health Professions

ABSTRACT

Background: Cancer patients are treated with a combination of cancer surgery, radiation therapy, chemotherapy, or other cancer therapies. However, most cancers cannot be cured. Immunotherapy is a novel treatment modality stimulating the body's natural immune defenses in order to fight cancer more efficiently. Previous studies show several human cancer types overexpress a molecule called Toll-like receptor 5 (TLR-5). A bacterial protein called Flagellin is a ligand to TLR-5 and can inhibit cancer cell development. However, it is not clear if TLR-5 is overexpressed in human prostate cancer cells or if Flagellin can be used as a therapeutic candidate for human prostate cancer. **Methods**: We performed RT-PCR to determine the expression of TLRs within the human prostate cancer cell lines LNcaP and DU-145. We further investigated the expression of TLR-5 within patient specimens through immunohistochemistry. The activation of TLR-5 signaling was investigated by quantitative RT-PCR detection of cytokine TNF-α within human prostate cancer cells. **Results**: We have found TLR-5 predominately expressed within different human prostate cancer cell lines and within different patient specimens. Furthermore, the bacterial protein Flagellin can induce a robust immune response in prostate cancer cells. **Conclusion**: Flagellin-based therapies may be a promising immunotherapy strategy used to prevent and treat human prostate cancer.

INTRODUCTION

Previous studies show several human cancers types (i.e. breast cancer tissues) overexpress Toll-like receptor 5 (TLR-5). Strikingly, the bacterial protein Flagellin, an agonist for TLR-5, can activate TLR-5-dependent signaling pathway and subsequently induce pro-inflammatory cytokines like TNF-α, resulting in cancer apoptosis and cancer regression (**Figure 1**).

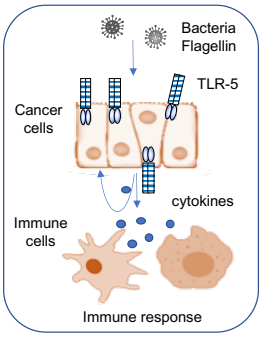

Figure 1. TLRs-induced anti-tumor immunity

A Flagellin-based therapeutic molecule named "Entolimod" has been evaluated in clinical studies. 26 patients with colorectal, non-small cell lung, anal, and urothelial bladder tumors were recruited. The clinical results show eight patients could maintain disease stability for greater than six weeks. Hence, Flagellin may be a promising therapeutic candidate.

However, previous studies have not cited TLR-5 as a therapeutic target for prostate cancer. Within mouse models, normal prostate tissue demonstrates strong TLR5 expression. When prostate tumor progresses, the expression level of TLR-5 decreases in both prostate tissues and in tumor tissues. Critically, a need arises to investigate TLR-5 expression within human prostate tumor tissues.

RESULTS

Human prostate cancer cells predominantly expressed TLR-5

To identify the TLRs expression profile on human prostate cancer cells, we first checked the expression of TLRs (TLR1-10) in human prostate tumor cell lines through RT-PCR via the primers listed in **Table I.**

TLR	Forward primers	Reverse primers
1	CGTAAAACTGGAAGCTTTGCAAGA	CCTTGGGCCATTCCAAATAAGTCC
2	GGCCAGCAAATTACCTGTGTG	CCAGGTAGGTCTTGGTGTTCA
3	ATTGGGTCTGGGAACATTTCTCTTC	GTGAGATTTAAACATTCCTCTTCGC
4	CTGCAATGGATCAAGGACCA	TCCCACTCCAGGTAAGTGTT
5	CATTGTATGCACTGTCACTC	CCACCACCATGATGAGAGCA
6	TAGGTCTCATGACGAAGGAT	GGCCACTGCAAATAACTCCG
7	AGTGTCTAAAGAACCTGG	CTTGGCCTTACAGAAATG
8	CAGAATAGCAGGCGTAACACATCA	AATGTCACAGGTGCATTCAAAGGG
9	TTATGGACTTCCTGCTGGAGGTGC	CTGCGTTTTGTCGAAGACCA
10	CAATCTAGAGAAGGAAGATGGTTC	GCCCTTATAAACTTGTGAAGGTGT

Our analyses identified several TLRs including TLR-1, TLR-3, TLR-5, TLR-6, and TLR-9, which are expressed in LNCaP and DU-145 cells (**Figure 2**). Of particular interest was the strong expression ofTLR-5 in both LNCaP and DU-145 cells.

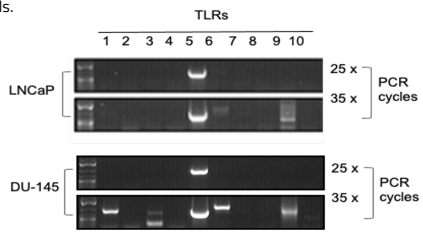

Figure 2. The expression profile of toll-like receptors (TLR) in human prostate cancer LNCaP and DU-145 cells. Both LNCaP and DU-145 prostate cells predominantly express TLR-5 as determined by a reverse transcription polymerase chain reaction (RT-PCR).

TLR-5 is up-regulated in human prostate tumor specimens

The finding of increased TLR-5 up-regulation in human prostate cancer cells LNCaP and DU-145 led us to investigate whether TLR5 is also up-regulated in patients with prostate cancer (**Figure 3**).

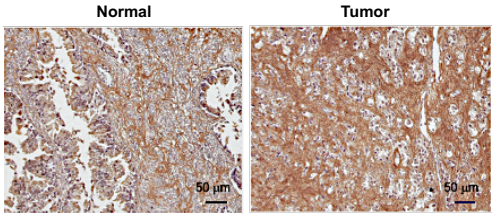

Figure 3. Toll-like receptor 5 (TLR-5) expression in human prostate cancer. Human prostate cancer tissue arrays were subject to immunohistochemistry analyses with monoclonal primary antibodies to TLR-5 as well as subject to biotinylated second antibodies. Brown color indicates positive staining for TLR-5. Representative images from tissues with different histological types are shown above: low TLR-5 expression in normal human prostate tissue; strong TLR-5 expression in prostate cancer tissue. Magnification. X40.

Flagellin activated TLR-5 signaling in human prostate cancer cells

We then explored the TLR-5 function in human prostate cancer cells by stimulating the prostate cancer cells with bacterial protein Flagellin (**Figure 4**).

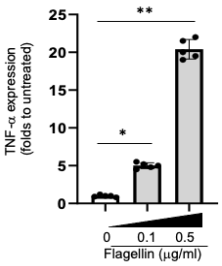

Figure 4. The expression profile of TNF-α by Flagellin-activated prostate cancer DU-145 cells. DU-145 cells were treated with 0, 0.1, or 0.5ug/ml Flagellin cultured for 24 hrs. DU-145 cells were harvested and the total RNA was isolated. Flagellin induced TNF-α was expressed by DU-145 cells as determined by real-time quantitative RT-PCR.
**P<0.05 untreated vs 0.1 ug/ml Flagellin.*
*** P<0.05 untreated or 0.1 ug/ml flagellin vs 0.5 ug/ml Flagellin*

CONCLUSION

- Our study demonstrated that TLR-5 was strongly expressed in human prostate cancer cells such as in LNCaP and DU-145 cells
- TLR-5 expression was up-regulated in the human prostate tumor specimen while normal prostate tissues expressed low levels of TLR-5
- Our study further demonstrated that Flagellin activated the human prostate cancer cell DU-145 by inducing TNF-α expression, which suggests the human prostate cancer cell is susceptible to the Flagellin treatment modality
- This study supports Flagellin-based therapies as a promising immunotherapy strategy for human prostate cancers
- The outcome of this study has significant implications for the design of future immunotherapeutic strategies in treating prostate cancer.

ACKNOWLEDGEMENTS

The author thanks Dr. Dekai Zhang affiliated with the Center for Infectious and Inflammatory Diseases at Institute of Biosciences & Technology, Texas A&M University for its material support and study supervision. This study is sponsored by AP Science Instructor Zachary Friske at DeBakey High School for Health Professions.