IKBKE INHIBITION- A TREATMENT FOR ADVANCED PROSTATE CANCER?

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Abstract:

This project investigated the role of novel threonine/serine kinase IKBKE– an enzyme- in androgen receptor regulation. In Prostate Cancer the androgen receptor acts as a 'cellular switch'. Interaction between the hormone testosterone and Prostate Cancer androgen receptors stimulates growth of the Prostate Cancer cells. Current therapies turn off the androgen receptor 'switch' causing the tumour to shrink. Unfortunately, the androgen receptor often develops resistance to these treatments by becoming 'switched on' in the absence of testosterone due to mutation.

Recent studies suggest that androgen receptor signalling may also be prevented through targeting an alternative cellular component—IKBKE. By understanding the role of IKBKE in Prostate Cancer cell development we can determine if it has the potential to be a valid drug target in advanced Prostate Cancer treatment.

KEY WORDS: Prostate Cancer, Androgen receptor, IKBKE, Serine/threonine kinase signalling, NFκB, Post-translational modification

Introduction:

Cancer of the Prostate Gland is the **most commonly diagnosed cancer in men**, **1 in 8 men will be diagnosed** in their life time. **128 men are diagnosed per day** in the UK.

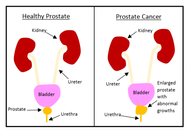


Figure 1– A healthy prostate versus a cancerous prostate.

Prostate cancer cells require the **androgen receptor to be activated in order to grow** at all stages of the illness. Current therapies involve **preventing androgen receptor signalling**. In advanced forms of the disease **Prostate Cancer cells develop resistance to therapy** by acquiring changes such as androgen receptor overexpression that result in **reactivation of the receptor**. It is therefore important that novel approaches are developed to treat Prostate Cancer to avoid and combat resistance to current therapies.

In addition to androgen, a **variety of co-regulatory proteins also regulate the activity of the androgen receptor**. For example, **the AR can be phosphorylated by a number of protein kinases (enzymes).** However it is unclear which kinases are involved in AR activation in advanced and therapy resistant Prostate Cancer.

A siRNA kinome screen identified a number of kinases which could play a role in androgen sensitive and resistant Prostate Cancer cell growth. The kinase **IKBKE** plays an **important role in AR transcriptional activation** and was the focus of my project.

Aims:

This project aimed to investigate the effect of pharmacological antagonism and siRNA knockdown of IKBKE on prostate cancer cell proliferation, colony forming ability and migration. By observing the effect of preventing the activity of IKBKE we hoped to determine if the kinase has potential to be a future therapeutic target in advanced Prostate Cancer treatment.

Materials and methods:

IKBKE siRNA knockdown

A combination of three siRNA's were used to knock down IKBKE in Prostate Cancer cell lines . The siRNA prevented the function of the IKBKE protein so the protein's function in the cell could be determined.

1) A 'pool' of various siRNA molecules that specifically bind to IKBKE mRNA were added to Prostate Cancer cells. Cells from the LnCAP and LnCAP AI Prostate Cancer cell lines were used.
2) The siRNA binds to the IKBKE mRNA . This prevents the production of IKBKE protein.
3) Cells were left to incubate at 37°C . The growth of the cells was monitored and compared to control cells treated with non-silencing (NS siRNA). NS siRNA does not prevent the transcription of any genes therefore the IKBKE protein should be produced in cells treated with NS RNA. Confluence—an estimate of adherent cells per well– was measured using the Incucyte Zoom. The Incucyte Zoom photographs cells regularly as they incubate to measure changes in confluence.
4) A colony formation assay was also performed to determine if the prevention of IKBKE activity could effect the ability of Prostate Cancer cells to form colonies.

Drug treatment:

Prostate Cancer cells were treated with the following IKBKE inhibiting drugs:

- **Bx795**- a TBK1/IKBKE and PDK1 inhibitor
- **Cyt387**- inhibits JAK1/JAK2 kinases and TBK1/IKBKE
- **Mrt67307**- TBK1, MARK1-4, IKBKE and NUAK1 inhibitor
- **Cay10576**- inhibits IKBKE only

The effect of the inhibitors was monitored using an SRB assay. This experiment aimed to determine the inhibitor that was most effective at reducing Prostate Cancer cell growth but was also suitable for use in a future study involving mice. Tumours made of LnCAP cells are known to be difficult to induce in mice, we therefore repeated the experiment in a different Prostate Cancer cell line, the CWR22rV1 cell line. CWR22rV1 tumours are more easily induced in mice than LnCAP tumours.

Investigating the role of IKBKE in Prostate Cancer cell migration

400,000 LnCAP cells were seeded and grown in full media for 72 hours . The layer of confluent LnCAP cells formed in the plate was scratched at regular intervals. The cells were then allowed to grow in the Incucyte Zoom where the migration of the cells into the scratch was monitored.

One plate was treated with DMSO, the other was treated with IKBKE inhibitor CAY10576. The migration of the differently treated cells into the scratch was then monitored to see in IKBKE has a role in Prostate Cancer cell migration.

Determining the position of IKBKE's effect in the cell cycle:

Previous analysis suggested that preventing the activity of IKBKE caused cells to remain in the G1 phase of the cell cycle and not progress into S phase. To confirm this cell cycle analysis was performed in IKBKE knockdown cells and cells treated with NS siRNA for comparison.

Results- Cell cycle analysis of IKBKE knock down LnCAP cells

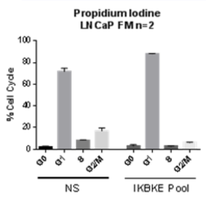
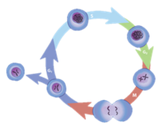
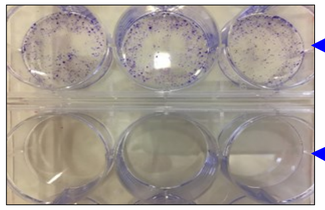


Figure 8- Analysis of LNCaP cells showed that knocking out IKBKE caused more cells to be stalled in the G1 phase of the cell cycle compared with cells treated with NS RNA. This confirmed the hypothesis that IKBKE effects the G1/S phase transition.



Results:

IKBKE knockdown blocks Prostate Cancer cell growth and colony formation



NS RNA – 5000 cells per well were seeded. Cells were treated with non-silencing siRNA. IKBKE was therefore not inhibited and many colonies formed.

IKBKE POOL siRNA – 5000 cells per well were seeded. In the cells which had IKBKE knocked out no colonies were produced suggesting that IKBKE is essential for Prostate Cancer cell proliferation.

Figure 2– Figure 1 shows a photograph of one of the repeats on the colony formation assay in the LnCAP cells. The same amount of cells were seeded in each well however the wells with the IKBKE knockdown (bottom row) no colonies were produced. In other repeats some colonies did form from the knock down cells however there was very few.

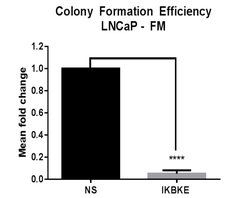


Figure 3- The colony formation assay was repeated 3 times in the LnCAP cells with full media (FM) . The average fold change in colonies across all 3 repeats is shown in figure 2.

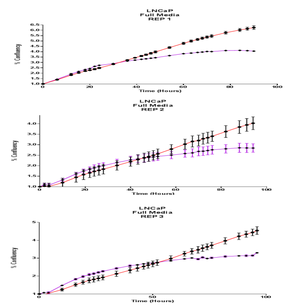


Figure 4– Figure 3 shows the 3 repeats of measurements of confluence made using the Incucyte Zoom. The red lines show how the confluence increased over time in the NS cells. The pink lines shows the effect of the addition of a pool of IKBKE targeting siRNA. All three repeats show a decreased confluence over time in cells treated with the IKBKE targeting siRNA.

Chemically inhibiting IKBKE blocks Prostate Cancer cell growth

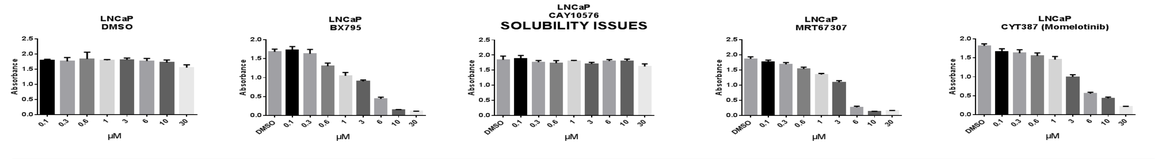


Figure 5- Shows the effect of various IKBKE inhibitors on the proliferation of LnCAP cells. As concentration of inhibitor (μM) was increased the absorbance decreased indicating that as the concentration of drug increased (as more IKBKE was inhibited) less LnCAP cells were able to survive. The exception to this trend was the cells treated with CAY10576. This is because the drug was not very soluble and therefore could not act to inhibit IKBKE.

Chemically inhibiting IKBKE blocks Prostate Cancer cell migration

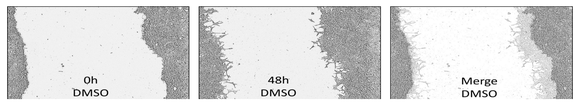


Figure 6– images taken by the Incucyte Zoom of the same scratch at different time points when treated with DMSO– a control substance. The third image shows the 2 images merged together, demonstrating the extent of which the cells have migrated.

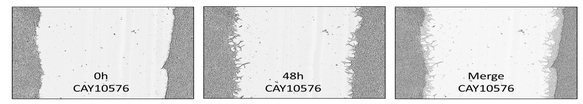


Figure 7– images taken by the Incucyte Zoom of the same scratch at different time points when treated with CAY10576– a drug that inhibits IKBKE. The third image shows the 2 images merged together, demonstrating the extent of which the cells have migrated. The migration of the cells is decreased when treated with IKBKE inhibitors compared to the DMSO control.

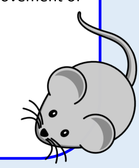
Conclusions

Results suggest that the kinase IKBKE plays an essential for Prostate Cancer cell survival. When the activity of IKBKE was prevented through an siRNA knockdown few colonies of Prostate Cancer cells were able to form compared with those treated with NS siRNA (figures 2-3). The proliferation of Prostate Cancer cells was slower when IKBKE was knocked out (figure 4) further supporting the idea that IKBKE is involved in Prostate Cancer cell survival. Similarly when IKBKE activity is chemically inhibited (figure 5), Prostate Cancer cell growth is supressed.

IKBKE may also play a role in Prostate Cancer cell migration as when its activity was inhibited using CAY10576 less migration was observed than when the cells were treated with DMSO (figure 6-7). If IKBKE inhibition is able to supress Prostate Cancer cell migration then this may indicate IKBKE's involvement in metastases (the movement of tumour cells).

FUTURE WORK

The next steps in this project will be to work out the effect of IKBKE inhibition in a living model such as a mouse. IKBKE knockdown CWR22rV1 tumours will be induced into mice and the disease progression compared to mice with normal CWR22rV1 tumours. From this we will be able to confirm IKBKE's role in tumour cell migration to see if it could be a potential drug target for Prostate Cancer treatment in the future.



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