Investigating the effects of *Naja nigricollis* venom on colorectal and pancreatic cancer cell lines to identify biomarkers to aid cancer treatment

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Abstract

Cancer is one of the leading causes of death worldwide, with both colorectal and pancreatic cancer related deaths rising year upon year. Due to the problematic nature of both diseases, they are difficult to diagnose and treat, and so scientists look to the natural world for novel leads to aid the progress of treating these diseases. This preliminary study investigates the effect of *Naja nigricollis* venom on both colorectal and pancreatic cancer cell lines, BxPC3 and SW620. The secretome of both cell lines were analysed, with results indicating some interesting effects of the venom on both cell lines. These effects may target specific signalling pathways, which may be vital in the treatment of these cancers.

Background

Pancreatic cancer is one of the leading causes of cancer related deaths worldwide, with colorectal cancer being one of the most common malignancies. Novel biomarkers are currently being investigated, with the aim that they will aid early diagnosis, to improve prognosis of the diseases.

Tumours in the pancreas tend to be aggressive, with resistance to treatments identified in bot pancreatic cancer and colorectal cancer. Metastasis affects both cancers, and as such make them difficult to treat.

Naturally available compounds have diverse structures and molecular content, and so possess precise biological functions. Secretions and venoms from arthropods, reptile and amphibians have shown antifungal, antimicrobial and antioncogenic effects. Thus, venoms could influence cell secretomes, which could identify specific signalling pathways activated and targeted by the cancer.

Research Objectives

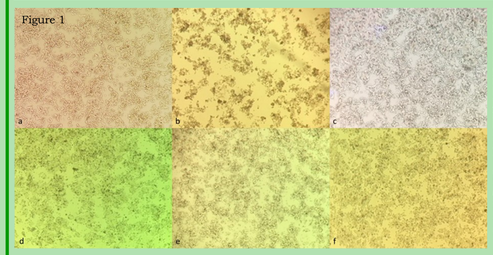
The main research objectives for this preliminary study into the effects of venom on pancreatic and colorectal cancer were:

⇒ To identify the effect of *Naja nigricollis* venom on pancreatic and colorectal cancer cells

⇒ To establish whether the venom affects the growth or viability of the cells

⇒ To analyse the secretions made by the cells and identify changes in the expression of proteins

Results



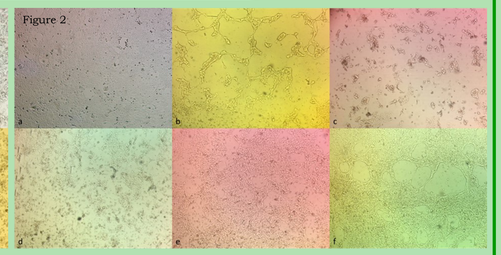
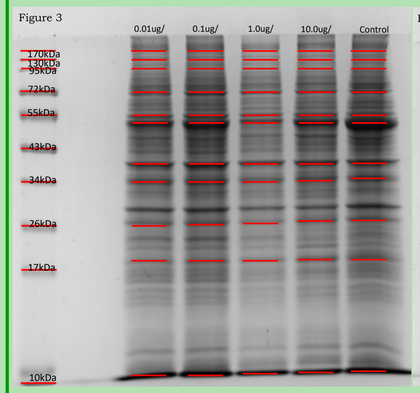


Figure 1: Response of CRC SW620 cells to varying concentrations of *Naja nigricollis* venom. a. before venom treatment, b. after treatment with venom at 0.01ug/ml, c. after treatment at 0.1ug/ml, d. after treatment at 1.0ug/ml, e. after treatment at 1.0ug/ml, f. control group, no venom. **Figure 2:** Response of PC BxPC3 cells to varying concentrations of *Naja nigricollis* venom. a. before venom treatment, b. after treatment with venom at 0.01ug/ml, c. after treatment at 0.1ug/ml, d. after treatment at 1.0ug/ml, e. after treatment at 1.0ug/ml, f. control group, no venom.

Morphological changes can be seen in cells which have been treated with venom following 24 hours of incubation, with a greater effect seen with the pancreatic caner cells BxPC3.



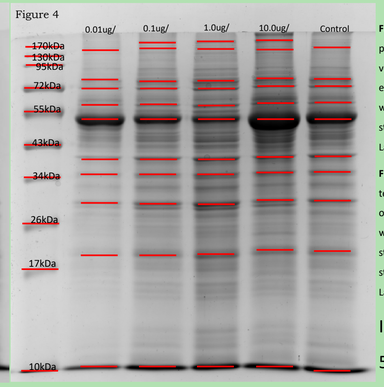


Figure 3: SDS-PAGE analysis of SW620 protein secretions after treatment with venom. 15ug of protein was loaded into each well. Lanes are labelled, and the gel was stained with Coomassie Brilliant Blue stain. Analysis performed using Image Lab[12].

Figure 4: SDS-PAGE analysis of BxPC3 protein secretions after treatment with venom. 15ug of protein was loaded into each well. Lanes are labelled, and the gel was stained with Coomassie Brilliant Blue stain. Analysis performed using Image Lab[12].

Intense bands close to 55kDa could indicate the presence of glycosylates, which have previously been identified in colorectal cancer cells. Bands at 72kDa could represent transforming growth factor receptor beta, known to be present in pancreatic cancer cells.

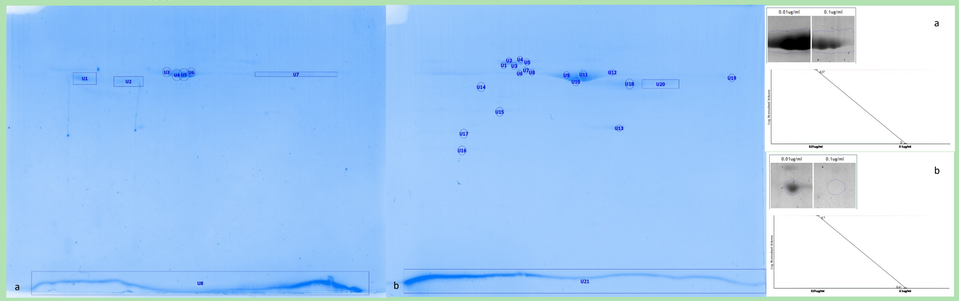


Figure 6: Image of 2D gel electrophoresis for the BxPC3 cell line at 0.1ug/ml (a) and 0.01ug/ml (b). Spots and smears manually labelled using Image Lab[12] software. Spots of interest shown from secretome of BxPC3, with their Log Normalised Values. Due to the lack of repeats and gels of sufficient and consistent quality, the results of the study are limited. Thus, differences seen may not reflect true variances.

Methodology

⇒ Two cell lines were selected: BxPC3, a pancreatic cancer cell line (Cancer Research UK, Manchester) and SW620 a colorectal cancer cell line (Professor Michelle Garret, University of Kent, Canterbury).

⇒ Cells were cultured in their respective media, counted with a haematocytometer, and plated out in T25 flasks.

⇒ 1ml of venom in media was administered to the cancer cells at varying concentrations; 10ug/ml, 1.0ug/ml, 0.1ug/ml and 0.01ug/ml, for two hours at 37°C , 5% CO_2.

⇒ Cells were washed with PBS, and left for 24 hours with 1ml media at 37° C , 5% CO_2. Media was collected and concentrated via TCA precipitation

⇒ Samples were run on 12% SDS-PAGE gels and analysed using Image Lab.

⇒ Samples were then run on IPG strips (first dimension) and then on 12% acrylamide gels (second dimension), and were analysed using Image Lab and SameSpots.

Discussion and Conclusions

⇒ Preliminary investigation showed positive results, the changes in morphology of both cancer cell lines can be identified

⇒ Changes in proteomic expression can be seen from the SDS-PAGE gel analysis, bands of varying intensities can be identified, with possible evidence of glycosylates at around 55kDa and Transforming Growth Factor Receptor Beta at 72kDa

⇒ 2D gel electrophoresis showed changes in the presence or absence of various proteins, by depicting intense or faint spots

⇒ Heat Shock Proteins may be present, shown through the increasing intensity of some spots. These proteins are known to be involved with both cancer cell lines, and play a role in metastasis, apoptosis, proliferation and differentiation

⇒ Intense large band along the bottom of each gel suggests the presence of smaller polypeptides, and the need to run larger gels

Future Study

⇒ Optimisation of most aspects of practical work is necessary, from collecting the secretome, to concentrating the protein and loading the protein consistently across all gels

⇒ Better saturation of protein is necessary to ensure clear results

⇒ Optimisation of the isoelectric focusing step, and repeat 2D gels, making analysis reliable and statistically valid

⇒ Mass Spectrometry should be used to identify proteins

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