Can deep-sea bacteria fight cancer?

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What is the problem?

Cancer, a condition where abnormal cells divide and grow in an uncontrolled way to form a tumour, is among the leading causes of death worldwide. It has been estimated that, in the UK, there is a 50% chance that an individual will be diagnosed with cancer at some point in their lifetime. Cancer can affect any part of the body which has living cells and a cancer may spread from one part of the body to another (see Figure 1).

Treatments for cancer include radiotherapy, surgery and chemotherapy, the latter involves the use of small molecules (cancer drugs) to kill cancer cells. A major problem with chemotherapy is that cancer cells can become resistant to cancer drugs, which then lose their effectiveness, resulting in the cancer cells still being able to divide uncontrollably.

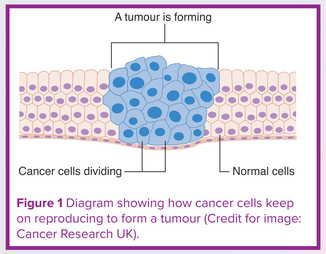

Figure 1 Diagram showing how cancer cells keep on reproducing to form a tumour (Credit for image: Cancer Research UK).

Due to this, researchers are trying to find alternative, novel chemical compounds that can be turned into cancer drugs. Natural products, particularly derived from marine microorganisms, are an outstanding source of possible drugs.

What are we interested in?

A group of candidate molecules, proximicins A, B and C (see Figure 2), with remarkable anti-cancer and antibiotic activities, have been extracted from a deep sea actinomycete bacterium, called *Verrucosispora maris* (see Figure 3). Proximicin B is more cytotoxic (toxic to living cells) compared to the other family members, causing cells to die. The way these interesting compounds work is not known and the aim of my project was to discover what molecules or proteins proximicins might be interacting with (their targets). Our final goal is to elucidate their mechanism of action.

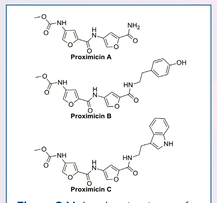

Figure 2 Molecular structures of proximicins A, B and C.

Figure 3 Actinomycetes bacteria.
Credit Oregon Caves from Cave Junction, USA. https://commons.wikimedia.org/wiki/File:Actinomycetes_bacteria_(10332880924).jpg

What did we do?

Using synthetic organic chemistry techniques, we made proximicin B in the lab from methyl-2-furoate (see Figure 4). We purified the products of the reaction using a chromatography technique, which separates the different components of a mixture in to their constituent parts. We then used a technique called Liquid Chromatography-Mass Spectrometry (LC-MS) to characterise the purified proximicin B and to confirm its molecular weight (see Figure 5).

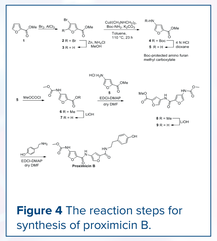

Figure 4 The reaction steps for synthesis of proximicin B.

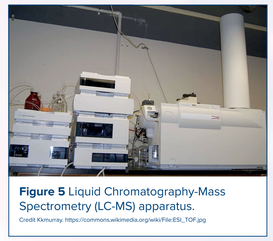

Figure 5 Liquid Chromatography-Mass Spectrometry (LC-MS) apparatus.
Credit Kmurray. https://commons.wikimedia.org/wiki/File:ESI_TOF.jpg

We then went on to try to find specific human target proteins to which the proximicins bound. We did this by identifying short sections of proteins (peptides) made up of 12 amino acids (the building blocks of proteins) that bound to the proximicins. We used a kit to do this which uses phages (a type of virus that infects bacteria) which have been engineered to produce lots of copies of different peptides. The phages 'display' the peptides on their surface and when a selection of phages displaying different peptides are added to the proximicins, the ones that bind can be selected and analysed further and the ones that do not bind can be discarded (see Figure 6). The particular phage display kit we used enabled us to test up to a billion different peptide sequences. We found 10 phages which displayed peptides which bound to the proximixins.

We wanted to determine the amino acid sequence of these peptides and we did this by extracting the DNA which coded for the peptides from the phages. We sent the DNA to a company called Eurofins, which sequenced the DNA for us, i.e. determined the order of A, C, G and T DNA bases in the samples.

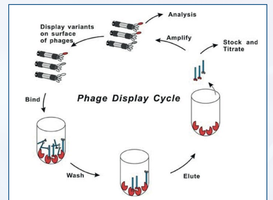

Figure 6 The process used to screen a phage display peptide library.
Credit "Creative Biolabs". https://commons.wikimedia.org/wiki/File:Phage_display_cycle.png

What did we find?

Of the DNA samples from the 10 phages that displayed peptides that bound to the proximicins, we received a DNA sequence (see Figure 7a) for 7 peptides, from which we were able to determine the peptides' amino acid sequence (a sequence of 3 specific DNA bases codes for particular amino acids, e.g. GTG codes for the amino acid called valine) (see Figures 7b and 7c). Six of the 7 phage displayed peptides had the same amino acid sequence, whereas the 7th had a different sequence.

We searched databases of the amino acid sequences of known proteins to establish whether the peptides corresponded to part of known proteins and we have identified a number of possible human protein-targets.

a

Phage 1	GTG CAT TGG GAT TTT CGG CAG TGG TGG CAG CCT TCT
Phage 8	GAT CGT TGG GTG GCT CGG GAT CCG GCG AGT ATT TTT

b

Phage 1	VHWDFRQWWQPS
Phage 8	DRWVARDPASIF

c

DNA sequence	Amino Acid	DNA sequence	Amino Acid
GTG	Valine (V)	TCT	Serine (S)
CAT	Histidine (H)	AGT	Serine (S)
TGG	Tryptophan (W)	CCT	Proline (P)
GAT	Aspartic acid (D)	CCG	Proline (P)
TTT	Phenylalanine (F)	GCT	Alanine (A)
CGG	Arginine (R)	GCG	Alanine (A)
CGT	Arginine (R)	ATT	Isoleucine (I)
CAG	Glutamine (Q)		

Figure 7 (a) DNA sequences from two of the phages that displayed peptides that bound to proximicin. (b) Peptide sequences of the two peptides we identified that bound to proximicin, determined (c) by using the consensus DNA and amino acid codes.

What does it mean?

We have found similarity regions between two peptides that bind to proximicins and previously known human proteins. We are currently establishing if the proteins identified are potential targets for cancer therapeutic intervention, and we are also planning other experiments to unravel the cellular signalling pathways that proximicins may modulate. As a result, this may pave the way for new anti-cancer drugs, based on natural marine products, with increased potency and anti-tumour activity in a broad range of cancers.

Who am I?

I am a 2nd year chemistry undergraduate student at the University of the West of Scotland. After I have completed my degree I hope to do a PhD or Masters in medicinal chemistry and eventually have a career in biomedical research.

Keir was supervised during his Vacation Scholarship project, *"Identification of the molecular targets of proximicins, potent anti-tumour natural products"* by Dr Federico Brucoli.