Collaborating to combat cancer

In the context of their successful partnership, Drs Csaba Szabo and Mark Hellmich highlight how scientists from different research backgrounds can come together to accelerate scientific progress and develop novel medical therapies in the fight against cancer.

Could you provide some background as to how you came to work together? Has collaboration accelerated the advancement of your research?

MH: Dr Szabo wanted to work on the role of H₂S in cancer and found our group on campus. I already knew of his work on H₂S but had no idea he wanted to expand into cancer biology. I feel that by uniting our expertise, we subsequently truly reached a new and exciting phase in both of our careers. As a direct result of the synergy of innovation that our collaboration has created, we have initiated several new lines of experimental and clinical investigations in the past year. We are also working very hard to find potential investors to support this work.

Dr Hellmich, as a seasoned expert in cancer cell growth, cancer microenvironment and metastasis, what has been your role in elucidating the influence of H₂S on cancer development?

MH: Prior to starting our collaborative studies, I worked on the interaction of the cancer cell and its environment in various contexts. I was always fascinated by the multiple layers of communication between the tumour cell and its environment (endothelial cells, fibroblasts and other cell types). Ultimately this interaction is what is so crucial for tumour growth and metastasis. I have conducted work in discovering new roles of a certain class of chemokine mediators and in the area of tumour stem cell biology, which is another key aspect on how tumours grow and induce metastasis. Thus, based on my prior work, I was not at all surprised when Csaba started to talk about the possible role of H₂S in the tumour microenvironment. I immediately recognised the potential importance of this mechanism, given the fact that H₂S is essentially a ‘local hormone’, with the advantage of being a gas, and able to diffuse in and out of cells and travel significant distances in the body.

You detected that colorectal cancer cells contain high levels of the H₂S-producing enzyme cystathionine-beta-synthase (CBS). By what means was this discovery unearthed?

MH: Myself and my surgical oncologist collaborator, Dr Celia Chao, had a collection of cancer tissues, and corresponding normal tissues in their freezer. These materials were collected from patients undergoing surgery in our University. Under our joint guidance, Dr Ciro Coletta, one of the postdoctoral fellows in Szabo’s laboratory, started to analyse these tissues for H₂S production and for the levels of the various H₂S-producing enzymes. Only one of the three H₂S-producing enzymes, CBS showed a marked increase in colorectal tumour tissue; the other enzymes were unchanged when compared to the non-cancerous colon tissue.

You are currently working to develop methods to block cancer cell pathogenesis with a view to translating this research for clinical use. Could you discuss some of the techniques being explored?

CS: We have expressed the human CBS protein in vitro and we are currently screening chemical libraries for potential inhibitors. There are also known ‘lead’ molecules, which we plan on optimising for cancer therapy. This is yet another direction of study we are working on right now. Once we identify a compound that is an effective inhibitor of CBS, with low toxicity, we will test its anti-tumour potential in mice bearing tumour tissue from humans. Some of this work will be conducted in the frame of a new spin-off company we have established, CBS Therapeutics. We are currently in discussions with potential investors to support this work.
The smell of success

A collaborative approach to understanding cancer has led researchers at the University of Texas Medical Branch, Galveston, to uncover a role for the characteristically pungent gas, hydrogen sulphide in the provision of energy to support cancer growth, cell proliferation and invasion.

HYDROGEN SULPHIDE (H₂S) is a colourless, flammable and hazardous gas with a distinct ‘rotten egg’ odour. It occurs naturally in petroleum, natural gas and hot springs and is released in large quantities during volcanic eruptions. Furthermore, it is often produced from the anaerobic bacterial breakdown of organic matter, leading to its more common names: ‘sewer gas’ or ‘swamp gas’. Perhaps surprisingly, considering its toxic nature, H₂S is also produced in the human body in small quantities by most cells. Three enzymes are known to synthesise H₂S: cystathionine-gamma-lyase (CSE), cystathionine-beta synthase (CBS) and 3-mercaptopyruvate sulphurtransferase (3-MST). It is synthesised from the substrate L-cysteine and, because of its gaseous nature, diffuses to its signalling target within the source cell or across cell membranes into nearby cells. By entering the bloodstream, H₂S can also affect remote sites in the body. Its signalling functions – via various mechanisms including the activation of potassium membrane channels, stimulation of kinase pathways and the inhibition of phosphodiesterase enzymes – induce blood vessel relaxation and angiogenesis; regulation of neuron communication in the brain; and stimulation of mitochondria – the energy producing organelle of the cell.

Dr Csaba Szabo, Professor of Anaesthesiology, has been studying the role of H₂S in critical illness with his group at the University of Texas Medical Branch (UTMB), Galveston. A recent collaboration with colleague Dr Mark Hellmich, Professor of Surgery, Physiology and Biophysics, has uncovered that H₂S plays a key role in colon cancer metabolism. Their work, published last year in Proceedings of the National Academy of Science (PNAS), entitled ‘Tumour-derived hydrogen sulphide, produced by cystathionine-beta-synthase, stimulates bioenergetics, cell proliferation and angiogenesis in colon cancer’, raises the potential for developing novel therapies to target this serious disease.

CHANGING DIRECTION

The two researchers began their investigations to test the hypothesis that tumour cells were acting via vascular endothelial cell growth factor (VEGF) to stimulate the surrounding blood vessels to produce H₂S and thus induce the growth of new blood vessels to meet the needs of the growing tumour. In fact, what they found was that one of the H₂S-producing enzymes, CBS, was markedly elevated in colon cancer cells in comparison to the surrounding, non-cancerous tissue; resulting in increased H₂S production. This was demonstrated in Szabo’s laboratory by Dr Ciro Coletta, a postdoctoral fellow, who was asked by Szabo and Hellmich to compare the levels of H₂S-producing enzymes and H₂S in tissue collected from cancer patient biopsies and patient-matched healthy tissue. His experiments showed that, of the three enzymes, CBS was the only one that was elevated above background levels.
Following this significant finding, further in vitro experiments demonstrated that cultured colon cancer-derived epithelial cell lines also exhibited up-regulation of CBS and increased H₂S production compared to non-malignant colon cells.

**MECHANISMS OF ACTION**

In order to understand why the cancer cells are producing these significant amounts of H₂S and uncover the role of the signalling molecule, the collaborating labs carried out a series of in vitro and in vivo studies to determine its localisation, associating cellular components and functions. Through separating and isolating particular types of cell component and organelle – using a process known as cell fractionation – the researchers were able to demonstrate that a large proportion of the total cellular CBS was associated with the mitochondria in colon cancer cells, specifically to the outer mitochondrial membrane. The significance of this finding was supported by evidence that H₂S can stimulate mitochondrial function to increase the cellular energy production that is necessary for tumour cell growth, replication and survival.

Additional in vitro experiments carried out by the researchers highlight the importance of H₂S for tumour cell proliferation, migration and invasion. By using short-hairpin RNA sequences to suppress the expression of CBS in colon cancer-derived cell lines, and thus inhibit production of H₂S, the researchers significantly reduced cell proliferation and growth of tumours; an effect that was not replicated in cells from non-cancerous tissue. Conversely, engineered over-production of CBS resulted in an increase in the rate of cancer cell proliferation and invasion.

The accumulated evidence collected by the UTMB researchers has led them to conclude that the cancer cell-produced H₂S serves three functions: “First, it stimulates cell division, perhaps by increasing the activity of specific intracellular signalling pathways. Second, it serves as a fuel to feed cancer cell metabolism by providing the mitochondria with electrons necessary for energy production. Finally, H₂S was found to diffuse outside the tumour cells to promote the growth of new blood vessels to supply the growing tumour tissue,” explains Szabo.

**FROM BENCH TO BEDSIDE**

The results from the colon cancer studies suggest that CBS could represent a therapeutic target for anticancer drugs. Experiments carried out by researchers from Hellmich’s laboratory have demonstrated that pharmacologically inhibiting CBS activity almost completely blocked cancer growth by eliminating its fuel supply. The potential of this is clear: “The goal would be to block H₂S in the tumours, preferably with a non-toxic molecule that could be given orally to cancer patients,” enthuses Hellmich. However, this is not a straightforward endeavour as CBS activity does not only occur in cancerous cells but in other tissues and organs where it has important signalling functions. Therefore, the researchers are currently directing their studies toward identifying a safe limit of CBS inhibition that disrupts its cancer-promoting activity without affecting the normal H₂S-associated signalling pathways of other normal body tissues.

**POTENTIAL AVENUES FOR FURTHER RESEARCH**

Although the researchers’ experiments to date have enlightened them on the three ways in which CBS activity promotes cancer cell growth, the mechanisms by which these occur remain elusive. The collaborators are actively seeking funding to embark upon this line of investigation, as well as other pertinent research. For example, the phenomena whereby dogs are able to identify patients with cancer based on their smell is relatively well-known. The finding that the strong-smelling H₂S gas is elevated in the tumour cells of cancer patients could explain this long-questioned peculiarity. Over the last few years, research published by other research groups has been developing highly sensitive cancer diagnostic strategies that involve detecting increased H₂S levels in the urine or breath of cancer patients. This is another area of research that Szabo and Hellmich would like to explore – tools to detect elevated levels of H₂S that would enable appropriate patients to be matched up with CBS inhibitory therapies.

Armed with the knowledge that CBS-synthesised H₂S in cancer cells is essential for energy production to enable their division, growth and invasion of host tissue, Szabo and Hellmich have founded CBS Therapeutics, a startup company involved in research and development of CBS inhibitors to target colon cancer. “We are actively working on raising additional funds, both for future basic research and for the commercialisation and therapeutic translation of our concept for patient benefit,” Szabo concludes. While the collaborators recognise that these are very early days in the field of H₂S and cancer biology, they envision various accelerated paths for the successful clinical translation of their discoveries.