Zfra: a potential therapeutic for cancer

Professor Nan-Shan Chang explains how his thorough research into an important signalling protein – WW domain-containing oxidoreductase – and its peptide binding partner, Zfra, has led directly to his latest work developing a novel therapeutic approach against cancer.

How have your research experiences in molecular biology and medicine guided you to your current focus of study?

I studied biology at a premier university in Taiwan, and I later received a PhD in Immunology in the US. From immunology, I expanded my research interests towards protein chemistry and molecular biology. My laboratory was the first to clone the protein-coding gene WW-domain-containing oxidoreductase (WWOX) from mice. In 2000, we characterised its function in cell death and cancer suppression. This has made a significant impact in cancer research. At the same time, collaborations with two former classmates and neuroscientists – Professors Chun-I Sze and Shur-Tzu Chen – have led to major advances in deciphering the role of WWOX in the nervous system and neurological disorders.

Can you enlighten us with the history of your groundbreaking research into WWOX?

My group and those of Professors C Marcelo Aldaz and Robert Richards independently discovered WWOX. My team at the Guthrie Research Institute was the first to isolate and functionally characterise the Wwox cDNA from mice. We were looking for genes that enhance the function of tumour necrosis factor (TNF) in mice. We were looking for genes that enhance the function of tumour necrosis factor (TNF) in mice. We were looking for genes that enhance the function of tumour necrosis factor (TNF) in mice.

As one of the leaders in this field, what have been your most significant findings to date?

I feel we have established key concepts and made several fundamental findings concerning WWOX. We have found the apoptotic and tumour suppressor functions of human and mouse WWOX in vivo and in vitro, as well as a new signalling pathway involving TGF-β1, membrane hyaluronidase (Hyal-2) as a receptor, and WWOX as an effector. Within this pathway, we have discovered that the WWOX/MEK1 complex acts as a molecular switch for turning cancer cell death ‘on’ or ‘off’. In addition, we have established the role of WWOX in neurodegeneration, neuronal injury, neuronal differentiation and neural development.

How has the Zfra protein been shown to both suppress the growth of and induce death of cancer cells?

Zfra is a 31-amino-acid zinc finger-like protein that participates in the TNF signalling pathway. TNF is a toxic protein that kills many types of cancer cells. A short, seven amino acid version of Zfra can initiate an effective anti-cancer response via our newly discovered Z immune cell lineage (see figure on page 110).

The human WWOX gene is located on a common fragile site of chromosome 16q, named FRA16D. As the name implies, a common fragile site on a chromosome tends to break apart during mitosis. Because cancer cells divide quickly, this gene breaks apart easily in a tumour cell line. Ultimately, when cancer cells do not express WWOX, they become aggressive, migrate quickly and invade tissues and organs very effectively. The impact of this research is still being felt today as shown by the 330 publications on the topic, 37 of which are from my laboratory.

What is the mechanism of these Zfra peptides?

We have determined that Zfra binds the membrane Hyal-2 of spleen Z cells and instigates the Hyal-2/WWOX/Smad4 signalling pathway. This signalling induces generation of memory Z cells that can trigger an anticancer response in vivo.

Can you outline the most exciting research with WWOX and Zfra currently being conducted in your laboratory?

We are now able to mount an anticancer response with Zfra in both immune competent and deficient mice. That is, the immune cells in ‘immunodeficient’ mice can be activated. We hope that our developed Zfra and Z cells can be tested in cancer patients in the near future. WWOX is also now a known tumour suppressor – we have identified a specific segment in WWOX, which is shorter than Zfra, that is very potent in blocking cancer growth. This is therefore another interesting direction for our laboratory.
Individuals at the National Cheng Kung University, Taiwan, are applying their investigation into the oxidoreductase WWOX to cancer research, aiming to one day take the sting out of one of the leading causes of death worldwide.

**CANCER IS THE** lethal result of uncontrolled cellular replication and invasion of the surrounding tissues. For a tumour to develop, many fundamental genes and pathways in the cell must be lost or mutated. Indeed, cancerous cells must overcome numerous cell cycle checkpoints, growth repression signals and, ultimately, cellular death signals. It is unsurprising, therefore, that such cells contain mutations in genes and proteins that control these key pathways. Cancer research often focuses on targeting these proteins or their expression in some way.

One such important regulator is the WW domain-containing oxidoreductase, WWOX. This enzyme has long been a focal point for cancer researchers, including Professor Nan-Shan Chang at the National Cheng Kung University, Taiwan, who has honed in on this protein-coding gene for the past two decades. In fact, Chang and his team were among the first scientists to identify and clone the WWOX gene in 2000. Since then he has been working both on the biochemistry and molecular biology of the protein, particularly its role in cell signalling.

**IDENTIFYING THE SIGNALS**

Chang did not start his career directly studying WWOX; his PhD and early postdoctoral research focused on elucidating the structure and function of cell membrane hyaluronan and the enzymes that manipulate them, the hyaluronidases. He found that hyaluronidases enhance the ability of tumour necrosis factor (TNF) to kill tumours, a fact that researchers looking to enhance the effects of anticancer drugs have utilised time and again: “These laboratory findings have been explored in clinical therapeutics for cancer for many years,” Chang states.

This early work inspired Chang to start investigating the signalling events that occurred after hyaluronidase/TNF interaction. In a search for genes significantly affected by this interaction, Chang isolated WWOX as a candidate tumour suppressor in the cell. Soon after, Chang and his team located the gene on chromosome 16, at a particularly fragile site that is vulnerable to breakage during ordinary cell division. Indeed, in 30-50 per cent of breast and prostate cancers, one copy of the

**NEURODEGENERATION, WWOX AND ZFRA**

It is no secret that as lifespans across the planet have started to increase, there has also been an increase in Alzheimer’s disease. In fact, in 2010 researchers from the University of Edinburgh discovered that China had more people living with Alzheimer’s disease than any other country in the world. Looking to take on the looming age-bomb that is threatening to overwhelm the healthcare industry in coming decades, Chang and his colleagues from National Cheng Kung University examined WWOX and its role in neuronal development and diseases. “My group found that when the amount of WWOX protein is reduced in the brain, a spontaneous protein aggregation cascade occurs in a sequential manner, involving TIAF1, TRAPPC6A and SH3GLB2,” Chang shares. “The aggregation leads to breakdown of membrane amyloid precursor protein and generation of amyloid beta, fibrils and plaques.” This discovery is supported by the team’s studies showing significant occurrences of ataxia, epilepsy, neurodegeneration and death in less than a month among mice with WWOX deficiency. Most recently, cases of WWOX mutations and deficiency in humans have been shown to also lead to growth and mental retardation, neural disorders and early death.

Excitingly, Chang’s discoveries of the protein Zfra may also be of benefit to patients with Alzheimer’s disease. “Zfra rapidly induces aggregated protein degradation *in vitro* and *in vivo*, suggesting its potential role in therapy for Alzheimer’s disease and other neurodegenerative diseases,” Chang notes.
To apply findings about WWOX and Zfra to innate immune system.

In the course of this work, Chang and his team looked for compounds capable of interacting with WWOX in the hope of finding a way to target cancerous cells. In order to identify a tool to inhibit WWOX, they conducted a yeast two-hybrid screen – an experiment that can help identify protein-binding partners from a library of cDNA candidates. The screen found that Zfra, a zinc finger-like protein that regulates apoptosis and a naturally occurring short peptide, was one such binding partner. From this, the researchers also discovered that Zfra participates in TNF signalling and therefore is heavily involved in one of the apoptotic pathways in the cell. More specifically, exogenous Zfra is involved in the transduction of signalling through interaction with membrane hyaluronidase-2 (Hyal-2) and, downstream, WWOX and Smad4.

Ultimately, Chang and his team found that Zfra increased the expression of a particular type of immune cell never before identified (see figure). These cells appear to be responsible for conferring protection from cancer growth. In wanting to find the extent of Zfra activity against cancer, Chang took cells – which he has named Z cells – from the same lineage and exposed them in vitro to Zfra. He then injected these ‘activated’ Z cells into cancerous mice. “We found that Zfra provides a protection against cancer through its interaction with Hyal-2,” Chang explains. “Blocking one signalling pathway mediated through it and WWOX, activated a second. This second goes on to downregulate the activity of Hyal-2 and activate WWOX in a different manner.” Perhaps, the result of these signalling pathways is the activation of the cell’s ability to identify and initiate the destruction of cancerous cells.