Thyroid trials

Dr Salvatore Sciachitano is passionate about improving diagnosis for thyroid cancer. Here, he discusses the challenges of identifying this complex disease and the translational achievements of his research group.

THYROID CANCER IS a rare but serious disease. In 2010, it killed 36,000 people, an increase of 12,000 from 1990. Despite its growing prevalence, it remains notoriously difficult to diagnose. Often the first symptom is a painless lump, or nodule, in the neck, but fewer than 5 per cent of these turn out to be malignant.

Though survival rates are high for the cancer, early detection of malignancy is imperative, as the major treatment is removal of the thyroid gland, requiring an invasive and unpleasant surgical procedure. Accurately identifying malignancy before surgery could prevent unnecessary suffering for thousands of people. By elucidating the molecular basis of this disease, Dr Salvatore Sciachitano from the Department of Clinical and Molecular Medicine at the University La Sapienza is getting closer to making this a reality.

To begin, what is your professional background? How did your interest in thyroid tumours develop?

My interest in thyroid cell transformation and the diagnosis of thyroid cancer began during my PhD training. In clinical practice with thyroid patients I noted the frequent occurrence of thyroid nodules yet the rare occurrence of thyroid malignancy. Identifying malignancy in thyroid nodules has often been compared to looking for a needle in a haystack. Thus, searching for specific markers of malignancy at the DNA, RNA or protein levels in the preoperative evaluation of thyroid nodules has been a major aim of my research.

Your lab was responsible for identifying HIPK2 as a new tumour suppressor gene for thyroid cancers. Is work underway to translate this discovery to the bedside?

We discovered the role played by HIPK2 in thyroid tumourigenesis after identifying a novel pathway that allows p53 – the guardian of the genome – to direct damaged cells to apoptosis. In 2006, we demonstrated that p53 induces apoptosis by downregulating the expression of an anti-apoptotic molecule called Galectin-3 (Gal-3), and that this process takes place in the presence of activated HIPK2.

This led us to postulate that the absence of HIPK2 could explain the expression of p53, paradoxically found alongside overexpression of Gal-3 in well-differentiated thyroid tumours. This is precisely what we found in a group of thyroid tumours. HIPK2 therefore represents a new diagnostic marker and a potential new therapeutic target. Its clinical use is hampered by the low quality of the antibody currently available, but studies are ongoing to obtain a better antibody and to include HIPK2 in the Thyrotest, currently performed with Gal-3.

What do you consider to be the most significant achievement of your lab?

Our lab has demonstrated that a combination of clinical and experimental studies, performed on well-characterised clinical problems, can dramatically advance knowledge of the molecular basis of disease and provide an opportunity to translate experimental discoveries into clinical practice. We have improved competence, both in the clinical management of patients with thyroid disease, and in experimental molecular biology techniques, leading to new diagnostic tests and therapeutic targets. We hope that this combined approach, with the creation of a Thyroid Unit will help answer important questions, resulting in more accurate preoperative diagnoses and more appropriate treatments for thyroid cancer.

Differentiating diagnostics

Research led by the University La Sapienza and University Niccolò Cusano in Rome, Italy, is characterising the molecular basis of thyroid cancer and has the potential to prevent unnecessary removal of the gland.
research on thyroid cancer, both experimental and clinical. This began with investigations of a molecule that inhibits programmed cell death (apoptosis), called Galectin-3 (Gal-3). Apoptosis is a critical biological mechanism, particularly when considering cancer. When a cell's DNA becomes damaged and cannot be repaired, it is targeted to this self-destructive pathway. As Gal-3 inhibits this process, its overexpression is associated with tumour progression, particularly in thyroid cells.

However, Gal-3 does have a counter factor. It is downregulated by the tumour suppressor p53 protein, enabling the destruction of potentially harmful cells. Mutations in the p53 gene are found in almost half of all tumours and 15 per cent of malignant thyroid tumours. Knowledge of the link between the two has led Sciacchitano to hypothesise that p53 mutations may affect the expression of Gal-3.

Investigating this further, the team found a link between Gal-3 expression and p53 mutation in thyroid cancer. Their results showed that normal p53 downregulates Gal-3, while the mutated form stimulates its expression, inhibiting apoptosis and increasing chemoresistance.

AN UNDISCOVERED PATHWAY

Building on these revelations, the lab was able to reveal a hitherto unknown apoptotic pathway. In a paper published in *Molecular and Cellular Biology*, they indicated that p53 must be phosphorylated, on a specific residue, in order to repress Gal-3. The phosphorylating protein responsible is called HIPK2.

Using RNA interference (RNAi) techniques, the researchers demonstrated that HIPK2 is essential for p53-mediated Gal-3 repression and were able to piece together a novel apoptotic pathway (pictured).

Thyrostest represents one of the most accurate tests available to identify malignancy among indeterminate lesions. Identifying malignant thyroid nodules is a mammoth task. When you consider sub-clinical nodules (those that are not accompanied by any symptoms), prevalence in the population can reach almost 70 per cent. Fortunately, around 90 per cent of lesions are benign, but there is currently no reliable method of identifying these without an operation. Because it is found only in thyroid cancer cells, Gal-3 is an ideal marker to provide this identification.

Based on this principle, Sciacchitano helped to develop a form of thyroid immunoscintigraphy based on Gal-3. Following injection of a radiolabelled antibody, it is possible to view tumours using a mini-camera. In time, this *in vivo* imaging method may enable more precise referral to surgery, and could even be used to image other Gal-3 expressing tumours, including breast cancer.

**THYROTERT**

More recently, Sciacchitano set his sights on fine-needle aspiration (FNA)-cytology, the most common method to screen thyroid nodules. Despite its status as the gold-standard, it has many limitations, with some malignant tumours appearing similar to benign lesions, and diagnosis is often only possible after surgery.

There is therefore a clear need for ancillary methods to improve the accuracy of this technique. Exploiting recent advances in FNA-cytology that enable scientists to perform multiple tests on small numbers of cells, the lab developed and applied Thyrostest, a new Gal-3-based test to preoperatively characterise thyroid nodules. Today, Thyrostest represents one of the most accurate tests available to identify malignancy among indeterminate lesions.

The test is applied to thyroid cells obtained by FNA, using abnormal expression of Gal-3 as a marker of malignant transformation. In a prospective multicentre study, comprising over 500 patients, Sciacchitano’s group showed that by basing surgery on Gal-3 expression alone, a massive 71 per cent of unnecessary procedures could be avoided. His group is presently evaluating the clinical impact and cost-efficiency of Thyrostest, and are optimistic that the Gal-3 based test will prove an effective way to manage patients with indeterminate thyroid nodules.

**CLINICAL TRANSLATION**

Although the fundamental biology of thyroid disease is a key focus of Sciacchitano’s work, as a clinician, the implications for patients remain at the forefront of his mind. He was thus keen to translate his knowledge of Gal-3 into a diagnostic test, and in 2008, he did just that, contributing to the development of an innovative method of imaging thyroid cancer by targeting the molecule.