To begin, could you briefly outline the purpose of your studies?

Over the last decade it became evident that a large portion of the human genome encodes for genes that do not produce proteins – the commonly-assumed final product of gene expression. Our group works on the identification of these genes and tries to describe their function in association with cancer.

What are the advantages of setting up your research and experimentation in Lund University’s Department of Oncology?

There are many different advantages including: large biobanks with tumour samples collected during many years; close interaction with clinicians; and, above all, close collaboration with excellent experts in genomic studies and in breast cancer in particular. The Department also has access to state-of-the-art technologies in genomics, which is a requisite for the type of work we are doing.

Can you explain your methodology for investigating breast cancer?

Our approach is to screen tumour materials using genome-wide tools and then select interesting candidates for in depth functional analysis. This requires the interaction of different disciplines. We are using next-generation sequencing technology to identify small and long non-coding RNAs. We combine screening with extensive bioinformatic analysis and then more classical molecular and cellular biology.

Have your team made any unique or interesting discoveries regarding the role of non-coding RNAs in cancer development?

We have recently discovered the existence of 367 new human genes that had escaped previous identification. Among these, there are several that are interesting in the context of cancer. One of them is encoded from the same gene that encodes a protein: the Human Epidermal Growth Factor Receptor 2, also known as Her2 or ERBB2. The Her2 gene is one of the most important cancer genes and has been studied for many years. It is also widely used in cancer diagnosis and treatment. Despite having been closely studied, we can now show that Her2 encodes not only one but two genes: the Her2 protein and a non-coding RNA. In fact, it contains a microRNA gene named mir-4728 embedded in the larger protein coding part. We are now studying the function of this new gene, which we expect to be involved in cancer development in some capacity.

Could you offer an explanation of Dicer and its role?

In humans, one of the major classes of non-coding RNAs are the microRNAs. These genes produce RNAs that are sequentially chopped into smaller pieces by two enzymes, Drosha and Dicer. Dicer is the last step in the production of mature, functional microRNAs. This is the classical view of microRNA genesis. Using the example of a structured RNA – the vault RNA – we demonstrated that Dicer could in fact chop other RNAs to produce molecules functionally and chemically identical to microRNAs. We named these small microRNA-like molecules small vault RNAs (svRNAs).

In simple terms, can you explain how vaults are linked to resistance to chemotherapy?

Vaults were discovered by a group led by Leonard Rome at UCLA more than 20 years ago. The vault particles include the vault RNA. Many different groups observed that these organelles were present in larger numbers in cells that displayed resistance to chemotherapy. The mechanism, however, was never described. We showed that the svRNAs, functioning as microRNAs, can regulate the expression of CYP3A4, one of the most important proteins in pharmacology and toxicology. It is responsible for breaking down a very large number of chemicals in the body, including drugs. With this we described the first of several possible mechanisms that relate vaults to drug resistance.

Could you comment on some of the sequencing techniques that have been used by the project team and how successful they have been during your exploration of non-coding genes?

We are using Illumina sequencing. Next-generation sequencing technologies arrived at the right moment for our research. We were conducting classical cloning to screen the tumours but although successful – we identified the svRNAs using this technique – it was time-consuming and inefficient compared with next-generation sequencing. It would have been impossible to identify so many new human genes if we had still worked with classical cloning strategies.

What are you hoping will be the major applications of your research?

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Techniques for therapeutic manipulation of microRNA function are becoming available. If so, our finding could improve a large part of cancer diagnostics and therapeutics.
Exploring the role of non-coding regulatory RNAs in cancer, research at the **Lund University Canceromics Branch** has identified a cause of drug resistance and discovered hundreds of new genes. The researchers are now focusing on the contributions of one of these new genes in particular.

**HER2-POSITIVE BREAST CANCER**, a fast growing and aggressive form of breast cancer that tends to affect younger women, accounts for about 20 per cent of breast cancers. It is a variant that tends to be notably resistant to current treatments, which usually combine a drug called trastuzumab (Herceptin®) and chemotherapy; but even after successful treatment, there is a high risk of its recurrence.

Her2-positive breast cancer takes its name from the high level of expression of a protein called Human Epithelial Growth Factor Receptor 2 (Her2) in some breast tumours. Trastuzumab is an antibody treatment that targets and blocks Her2 function, by activating the immune system to suppress Her2 and so stop Her2-positive cancer cells from proliferating. Trastuzumab has been shown to reduce deaths from Her2-positive cancer by about a third and to reduce the risk of recurrence by about a half. As with all such treatments, however, there are side effects, the most significant being risk of heart damage, as trastuzumab also suppresses Her2 function in healthy tissue; and chemotherapy drugs similarly damage healthy tissues.

Most research into cancer over the last few decades has focused on protein-encoding genes, which account for less than two per cent of the human genome. More recently, however, it has been discovered that gene expression is also controlled by different classes of regulatory non-coding RNAs (nc-RNAs), which play a key role in cell differentiation, proliferation and growth. The paradigm for cancer research has accordingly evolved.

**NON-CODING GENES AND CANCER**

Her2 structure and function has been the subject of a great deal of study over many years, but recently Dr Carlos Rovira and his group in the Division of Oncology at Lund University have made a significant new discovery about Her2: Her2 codes a nc-RNA, in addition to coding the Her2 protein. Rovira posits that this new nc-RNA, named mir-4728, plays a role in the development of Her2-positive breast cancer and also in a number of other cancers.

Rovira attributes the unexpected discovery of mir-4728 as well as other key findings to the multidisciplinary and collaborative nature of his group’s research: “Collaboration bestows great benefit: through interaction with researchers with different expertise, with everyone contributing different ideas and solutions, we can set more ambitious goals than just simple descriptive characterisation of what we see. We hope that we will make a real contribution in this area,” he explains.

Rovira’s laboratory have so far identified hundreds of miRNA genes that had never before been recognised, more than 10 per cent of which they have mapped to genomic regions that are prominent in breast tumours. Rovira attributes the unexpected discovery of mir-4728 as well as other key findings to the multidisciplinary and collaborative nature of his group’s research: “Collaboration bestows great benefit: through interaction with researchers with different expertise, with everyone contributing different ideas and solutions, we can set more ambitious goals than just simple descriptive characterisation of what we see. We hope that we will make a real contribution in this area,” he explains.

Rovira began studying non-coding genes in the mid-1990s: “Then, the problem was to convince the scientific community that this was an important subject. Today, the situation is completely different, with thousands of people working in the field. Our knowledge is developing quickly but still the function of most microRNAs is completely unknown,” he states.

**CREATING NEW MIRNAS: SMALL VAULT RNAS AND DRUG RESISTANCE**

In exploring drug resistance in cancer, Rovira’s group identified as a subject for study a model organelle, known as the vault. Vaults are the largest organelles in cells and are classed as ribonucleoprotein particles. Their prime function is largely unknown, though they are thought to act as plugs in nuclear pores. They are shaped like a barrel and are largely hollow so they may act as carriers of material. Vault organelles are also present in greater numbers in tumour cells that show multi-drug resistance, including resistance to chemotherapy drugs: “The vaults are...
mysterious particles that are present in many eukaryotic cells," Rovira elucidates.

Rovira’s laboratory found that vault RNA is cleaved by Dicer, an enzyme that cuts up RNA strands, to create smaller strands that functioned as microRNAs (miRNAs), and named these as small vault RNAs (svRNAs).

The researchers found that the svRNAs that they had discovered regulate an enzyme known as CYP3A4, which is key in the process of metabolising drugs.

THE NEW MIR-4728 GENE

In Her2-positive breast cancer, Her2 and the ncrNA encoded within an intron of the Her2 gene, mir-4728, are both highly expressed. Rovira considers that the interaction of Her2 with mir-4728 may account for some poor results from cancer treatments that target Her2. His group is now analysing the expression profile of mir-4728 in relation to Her2 expression in tumour samples: "Over-expression of Her2 and the concomitant over-expression of mir-4728 is not only found in one in five cases of breast cancer, it is also present in ovarian, stomach, lung, uterine cancer and so on," he asserts. In breast cancer patients, testing for over-expression of the Her2 protein coding gene is routine for prognosis and for determining appropriate therapy, but not all patients respond equally well. The Her2 protein alone cannot always explain the reason why: “The non-coding mi r-4728 would probably account for the biology underlying this behaviour,” Rovira suggests.

KEY FINDINGS

Over the course of their work, using massive next-generation parallel sequencing techniques, Rovira’s laboratory have so far identified hundreds of miRNA genes that had never before been recognised, more than 10 per cent of which they have mapped to genomic regions that are prominent in breast tumours. A large proportion of the group’s candidate new miRNAs have shown association with Argonaute proteins, which they believe indicates that they are indeed new functional miRNA genes. The suspicion is that more remain to be found.

In addition to establishing that miRNA-like molecules can be created from non-miRNA precursors and identification of the role of svRNAs in furthering resistance to therapy, the research group have also found that breast tumour tissue differs from normal breast tissue in terms of expression levels of similar sets of miRNAs. Rovira feels that, taken together, their discoveries simply highlight the complexities of miRNA regulation, especially in cancer.

In the future, Rovira’s group intend to explore the new mir-4728 gene and its role in regulating and promoting breast cancer further. They aim to describe the function of mir-4728 and its involvement in cancer development: “Although we have parallel lines of investigation, we believe that greater knowledge of the mir-4728 gene will contribute most to our understanding of Her2 cancers and the role of the Her2 locus – and hopefully help patients with better treatment,” concludes Rovira.