LORNE CANCER CONFERENCE

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One of the major themes at the 13th Lorne Cancer Conference on 8-11 February 2001 was the ongoing search for the genes that predispose women to breast cancer. In other words, "after BRCA1/2". Presentations by Bruce Ponder (Cambridge Institute of Medical Research), Georgia Chenevix-Trench and Kum Kum Khanna (Queensland Institute of Medical Research) addressed this issue. John Hopper (University of Melbourne) and Mark Skolnick (Myriad Genetics, Salt Lake City) addressed the future challenges for breast cancer screening.

Another report story concerned the new finding that the paracrine hormone VEGF, secreted by tumours, recruits not only the vasculature but also lymphatic vessels. This suggests lymphoangiogenesis may be as important for metastasis as angiogenesis. Presentations from Kari Alitalo from the University of Helsinki and Steve Stacker from the Ludwig Institute in Melbourne are described below.

After BRCA1/2

The cloning of BRCA1 and BRCA2 was a tour de force, collectively representing some 500 person years. But for all that, these genes still only account for some 17% of hereditary breast cancer. That means the vast majority of high risk women in the population would be none the wiser for testing; a negative result for BRCA1/2 does not mean they are clear for other predisposing genes. But how does one nail the remaining genes?

According to Ponder, these genes are either going to be like BRCA1/2 – rare, but highly penetrant single mutations – or may be a constellation of weakly-acting gene variants or polymorphisms that creates the high-risk genotype. Such genes rather than being part of growth signalling or DNA repair pathways (like Ras, p53 or BRCA1) might influence ancillary processes like the connectivity of the intercellular matrix , immune surveillance, angiogenesis, or paracrine factors.

In Ponder's East Anglia study, he selected 29 candidate genes that might plausibly influence the way cancers develop or spread and looked at whether particular single nucleotide polymorphisms (SNPs) associated with these genes were more often associated with breast cancer cases than with controls. Overall he looked at SNPs in 3000 cases and several thousand controls, generating some 194,000 DNA samples.

Despite its size, so far the study has failed to reveal any major new breast cancer predisposition genes and has shown only a weak association for a few known genes. For instance polymorphisms in three genes showed an increased relative risk in cases versus controls BRCA2 (RR= 1.3), the paracrine hormone, TGFbeta (RR=1.4) and a gene involved in DNA repair, XRCC3 (RR=1.36). But as Ponder pointed out, even these associations were at the limit of statistical reliability. He believes much more needs to be known in terms of validating the SNPs; some may not even be usefully associated with the gene. And he says that this approach will probably only start yielding dividends when researchers don't make hunches about which genes will be important, but scan "the entire deck of cards". Such whole genome scans are still beyond anyone's budget, but new techniques are on the way.

Georgia Chenevix-Trench's presentation addressed the question of what role the ATM gene plays in hereditary breast cancer. She reported on results emerging from KConfab, an Australia-wide study of some 300 breast cancer families, 83 of which do not show mutations in either BRCA1/2. In collaboration with Kum Kum Khana, Chenevix-Trench examined whether mutations of the ATM gene (which underlies Ataxia Telangiectasia) may be involved. Current epidemiological evidence suggests that breast cancer is 5-7 fold more common in carriers of ATM, and according to one estimate (Swift et al) ATM heterozygotes could account for some 7% of breast cancer. Chenevix-Trench's question: is ATM a low-risk breast cancer gene, or a high-risk gene like BRCA1?

So far, studies have produced different findings. Daniel Haber at Dana Faber Institute failed to find a relationship between ATM protein truncation mutations and breast cancer cases relative to controls in the general population. But a recent study (Malcolm Taylor) of two Scottish families with a mild form of AT, but a 12-fold increased risk of breast cancer, revealed that they carried a missense allele of the ATM gene. A German family, carrying a mutation that produced a truncated ATM protein also showed an increased risk of breast cancer.

Chenevix-Trench reported that the Scottish mutation has been found in one of the KConfab families and segregates with breast cancer in this family. Five out of five affected members carry the mutation, as well as three unaffected members. Kum Kum Khanna's work (more below) has shown that this mutation creates a dominant negative protein, as evidenced by its ability to inhibit normal ATM kinase activity in the test tube. Further evidence that this mutation is dominant comes from studies of the tumour tissue in heterozygous individuals. Unlike BRCA1, where both copies of the gene become defective in tumours (loss of heterozygosity), there is no loss of heterozygosity in the ATM tumours. Two families were also found to carry an ATM protein truncation mutation. The significant incidence of ATM mutations in these breast cancer families (three out of 78) raises a dilemma. Since ATM mutations render cells less able to repair damage, should such families have frequent mammography or radiotherapy?

To see if less severe changes to the gene may also contribute to breast cancer, Chenevix-Trench and collaborators are also looking at the prevalence of an ATM polymorphism (TSer707Pro) in a population-based case-control study (1353 cases and 688 controls). So far no significant difference in the frequency of the polymorphism versus the more common allele have been found.

Kum Kum Khana described her team's focus on discovering what the ATM protein actually does. They have previously shown that ATM plays a key role in sensing and repairing DNA double-strand breaks. These molecular wounds are wrought by gamma radiation and oxygen free radicals, or they can be generated during the normal course of DNA replication or homologous recombination. Khanna has shown that ATM is a kinase (a member of the PI3 kinase family), an enzyme that phosphorylates its substrates. Some of these turn out to BRAC1, p53, Chk2 and Nibrin, all genes involved in either DNA repair or cell cycle arrest. ATM is probably part of a multi-component repair engine, but the evidence from Khanna's group suggests ATM is the driver. Just how ATM drives the

process remains to be worked out, but the phosphorylation and consequent stabilisation of p53 is probably the key. This process fails in ATM mutants.

ATM works in parallel with at least two other DNA damage-sensing proteins, ATR and DNA protein kinase (DNA PK). The different proteins appear to be triggered by different sorts of damage. ATR for instance is triggered by UV radiation (which causes pyrimidine dimers and single-strand breaks) rather then double-strand breaks. But to a large extent, DNA PK can cover for ATM. In ATM mutant cells 90% of double-stranded DNA breaks are repaired by DNA PK, but cells fail to arrest at checkpoints.

The link between the protein and its most extreme manifestation, Ataxia Telangectasia, is still enigmatic. Patients appear normal until about two years of age then show balance and walking difficulties, attributable to the deterioration of Purkinje cells in the cerebellum. Neurological effects remain paramount; they are usually wheelchair bound by eight or nine years of age and have a life expectancy only into the teens. This form of the disease is associated with protein-truncating mutations. On the other hand, missense mutations manifest differently. Individuals have microcephaly rather than ataxia and cancer is more prevalent. Knock-out mice on the other hand, fail to develop ataxia, but die of cancer after three to four months.

Khanna suspects that the impact of the dysfunctional ATM gene on breast cancer will depend on the type and location of the mutations in this huge (200kb) gene.

The carrier rate of ATM is 1%, and if the kConfab studies continue to show that carriers are at high risk of breast cancer, "that will have immense public health impact", says Khanna.

Mark Scolnick adressesed the brave new world of genetic screening. He made the point that most cancers have a strong family component. And while the past progress in identifying such predisposition genes has been painfully slow, that is likely to change in the coming era of high throughput methods for SNP analysis.

But Scolnick wonders whether society will be ready to accept the consequences – a windfall of genetic tests. The experience with testing for BRCA1/2, which Myriad genetics has now offered for several years, is that the test is underused. They test about 100 American women per week, less that 10% of those who could benefit, he says. The reluctance to test may reflect a fear of discrimination, but Scolnick says the perception of discrimination is greater than the reality. He points to laws passed since 1996 in several states that make genetic discrimination illegal. And he points to numerous interventions that could actually help BRCA1/2 carriers ranging from preventive use of tamoxifen to prophylatic oophorectomy.

John Hopper's presentation countered that this sort of public health advice needs to be tempered with the right statistics. Hopper's unique population-based study of breast cancer cases, controls and their families has indicated that the true risk from BRCA1 for women in the general population is significantly lower (40%) than that estimated from high-risk families (80%).

Lymphoangiogenesis

Once a tumour reaches a diameter of 2cm, it will suffer hypoxia. Most tumours start producing vascular endothelial growth factor, VEGF that binds to receptors on endothelial cells and coaxes them to grow toward the tumour and vascularise it. Since the tumour thus gets supplied both with a lifeline and transport, a major thrust of cancer research has been to find ways to block this process known as angiogenesis. But says Kari Alitalo, from the University of Helsinki, "nobody put the lymphatics into the picture". Though the lymphatic system has long been known to spread cancer, the flimsy, lymphatic vessels were not themselves thought to be able to penetrate into a tumour. Rather they were thought to enter the scene late in the piece to help mop up the fluid leaked by the ingrowing blood vessels.

However, recently a VEGF receptor, VEGFR3, was found to be expressed in lymphatic endothelial cells. Evidence that this receptor played an important role here came from the finding that a rare case of familial primary lymphoedema was shown to map to 5q 35, the locus of the VEGFR 3 gene. And mice treated with antibodies against the VEGFR3, also showed signs of oedema. Since tumour cells produce the ligands VEGFC and VEGFD, that bind to this receptor, the question has been whether these actually recruit the lymphatic endothelium to the tumour as well as blood vessels.

Kari Alitalo showed that introduction of VEGF-C into mouse models of pancreatic beta-cell tumours or breast carcinoma, stimulated the growth of lymphatic vessels around the tumours and metastasis. Adding a soluble form of the receptor, VEGFR-3, reversed these effects, presumably by preventing VEGF-C binding to its membrane bound receptor. Steve Stacker from Melbourne's Ludwig Institute found that VEGF-D when introduced into a slow-growing mouse tumour model (introduced into human 293 cells which normally lack VEGF family members and grow as xenografts in mice), stimulated the formation of lymphatics within the tumour as judged using Lyve-1, a marker for lymphatic endothelium, and the spread of the tumour to the lymph nodes. That effect could be blocked by an antibody specific to VEGF-D. On the other hand, introducing VEGF into the tumour did not stimulate lymphatic spread. The findings show that lymphatic vessels can be established in solid tumours and provide a route of spread. They also show that the particular VEGF made by the tumour can determine its route of metastatic spread. □