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MOLECULAR ONCOLOGY

The positive in the negative

Researchers are delving into triple-negative breast cancer, uncovering potential drug targets for this difficult-to-treat disease.

BY KENDALL POWELL

Rushing between meetings at the University of North Carolina at Chapel Hill, oncologist Lisa Carey scoffs at the current descriptor 'triple-negative breast cancer'. "Defining anything by what it isn't is scientifically insane. But, clinically, it's what we've got."

When patients are diagnosed with breast cancer, their tumours are classified into one of four pathological subgroups based on whether the tumours express the oestrogen receptor (ER positive) or the progesterone receptor (PR positive), overexpress one of the members of the epidermal-growth-factor receptor family (HER2 positive) or none of these — a classification known as triple-negative breast cancer (TNBC). When cancers are driven by the hormones or the HER2 pathway, the good news is that effective targeted therapies exist. For the remaining cancers, there are no clear targets.

For patients with TNBC, conventional chemotherapy, which affects a wide range of dividing cells, is the only available treatment after surgery and radiation. But this approach has met with mixed success. TNBC is often aggressive and highly resistant to chemotherapy. The five-year survival rate for TNBC is about 77%, compared with 93% for other types of breast cancer.

TNBC accounts for about 10–20% of all breast cancer in the United States (see 'The hard facts', page S50), with a higher incidence in certain populations. In African-American women, for example, it constitutes about 30% of all breast cancers[1]. And in women who carry an inherited mutation in the *BRCA1* gene, most breast-cancer cases are TNBC. It also tends to be more common in younger women.

MIXED COMPANY

The news is not all bad. A fraction of TNBC cases respond well to chemotherapy given before surgery, with some women even experiencing a complete response — that is, no evidence of live tumour cells at the time of surgery. And research into TNBC is still at an early stage — specific clinical trials for TNBC did not begin until 2006. To make headway against TNBC, however, researchers must first come to grips with the burgeoning data showing that this subgroup is heterogeneous.

Biologists are now beginning to divide TNBC into subcategories and to dissect the molecular signals driving each one, with the hope of finding targets for clinical trials. "We're acknowledging that this is not one disease, and we're designing trials with that in mind," says Jennifer Pietenpol, director of the Vanderbilt-Ingram Cancer Center in Nashville, Tennessee.

The trend of defining breast cancers by their molecular profiles (or gene-expression patterns) began in 2000, with the publication of a landmark study[2] led by Charles Perou, a cancer geneticist now at the University of North Carolina, Chapel Hill. In the past decade, researchers have defined six molecular subtypes of breast cancer, including two 'luminal' subtypes (which generally match ER-positive cancers) and a 'HER2-enriched' subtype (which matches HER2-positive cancers).

A significant proportion of TNBC, 50–75%, matches the molecular subtype known as 'basal-like' breast cancers[3]. These are characterized by the high expression of genes that are normally expressed in the basal epithelial layer of skin, such as the keratin-5, -6 and -17 genes. Most basal-like breast cancer is triple-negative; however, not all cases are, so the two terms are not fully interchangeable.

"If we are ever going to make therapeutic advancements in TNBC, we'll be targeting the 75% that is basal-like," says Perou. He adds that anything that helps TNBC patients in a clinical

trial must be working on this subset. "I always argue, let's track the biology — and the dominant biology is the basal-like subtype."

Others favour different approaches, noting that focusing solely on this basal-like subtype of TNBC might not be specific enough to yield broader therapeutic results. "We should look at each tumour individually," says John Carpten, a cancer genomicist at the Translational Genomics Research Institute in Phoenix, Arizona. "Even among basal tumours, growth can be driven by completely different molecular signals, so drilling down to the actionable driving events for individual tumours seems like a rational approach as well."

CELL BY CELL

Carpten's group is 'deep sequencing' TNBC tumours, which involves sequencing multiple cells within a tumour to identify coding mutations, deletions or translocations. His team will then merge this genomic information with whole-transcriptome data (representing all the expressed genes). The hope is to eventually incorporate the phosphoproteome: the set of proteins that is modified by addition of a phosphate group by protein kinases, thereby altering the proteins' activity in intracellular signalling pathways. This approach, he says, will provide doctors with a reference page for a patient's tumour that can point them to the drugs most likely to have the biggest impact.

The first published deep-sequencing effort[4] looked at 104 primary TNBC tumours and found a wide range of genetic mutations. Some tumours had just a few coding mutations, whereas others had hundreds. This analysis, led by molecular pathologist Samuel Aparicio at the BC Cancer Agency Research Centre in Vancouver, Canada, showed predominant mutations in well-known tumour-suppressor genes, such as the gene encoding p53 (called *TP53*), as well as in oncogenes. In several tumours, however, too few of the tumour cells contained these mutations for them to have caused the cancer. Affirming Carpten's sentiments, Aparicio's group found that basal-like TNBCs in fact showed more genetic variation at diagnosis than other TNBCs. "There's no more efficient way of learning that information about a tumour than sequencing it," says Aparicio.

The growth of ER-positive and HER2-positive cancers is largely driven by signalling through these receptors. Now researchers are attempting to determine whether basal-like cancers or, importantly, other TNBCs also share molecular drivers. "We need to define these triple-negative cancers positively by their drivers, but we aren't quite there yet," says Alan Ashworth, chief executive of the Institute of Cancer Research in London.

Until recently, the only candidates for defining TNBC were mutations in the *BRCA1* gene, the tumour-suppressor gene *TP53* and, occasionally, mutations or loss of the tumour-suppressor gene retinoblastoma 1 (*RB1*). Even this limited information has been put to use, though.

In 2005, Ashworth and his colleagues discovered the first evidence that BRCA1 loss might be exploited for TNBC therapy[5]. In a preclinical study, they described a combination of loss of BRCA1 and inhibition of the enzyme poly(ADP-ribose) polymerase (or PARP) that was lethal to tumour cells. DNA damage, such as breaks in the DNA, is caused by exposure to radiation, including sunlight and radiotherapy, and by the normal process of cell division. PARP is involved in the repair of single-strand breaks in DNA, whereas BRCA1 participates in double-stranded break repair. So BRCA1-deficient cells that also lack PARP activity cannot repair DNA damage and subsequently die. This effect was so persuasive that by 2007 PARP inhibitors were being trialled in patients carrying *BRCA* mutations.

The results of these trials have been mixed, however: *BRCA1*-mutation carriers responded well, but other TNBC patients did not. (The trial results have also been confounded by the inclusion of one drug, iniparib, which is unlikely to be a true PARP inhibitor.) "There's a heterogeneity here that we don't understand," says Carey. Maybe in TNBC that isn't driven by *BRCA1*, "you have to kick DNA-damage repair in another way, perhaps by hitting the tumour with direct DNA-damaging agents such as radiation or certain chemotherapeutics", causing unrepairable breaks.

Another approach is being taken by Bryan Schneider and Milan Radovich, cancer researchers at Indiana University in Indianapolis. They are looking for other weaknesses in TNBC cells by comparing the genomes and transcriptomes of TNBC tissue with those of healthy breast tissue. As tumour cells are dividing very rapidly, it was no surprise to find that *PARP* and genes in the *BRCA1* pathway are upregulated in TNBC samples. However, in as-yet unpublished research, Schneider and Radovich also discovered that a handful of protein kinases involved in epithelial-cell development and cell survival were also expressed at high levels. "We were very surprised to find that the top overexpressed kinases have not been studied in breast cancer at all," says Radovich — a result suggesting new drug targets.

CLASSIFYING THE UNCLASSIFIABLE

These investigations suffer from TNBC's heterogeneity, however. Mapping the genomic or transcriptomic differences from normal tissue is likely to reveal the dominant features of basal-like TNBC. But there is probably noise in these studies from the other, non-basal-like, TNBCs. Pietenpol's group has begun to tackle that problem, essentially by subtyping the subgroup — with striking results[6].

Pietenpol and her team searched for gene-expression data for as many cases of TNBC as possible. The team performed a cluster analysis on the resultant 587 tumours to identify gene-expression subtypes within TNBC.

This analysis found six distinct gene-expression profile clusters: basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory, mesenchymal, mesenchymal stem-like and luminal androgen receptor (LAR). As expected, the BL1 and BL2 subtypes dominated, making up about 50% of the TNBC samples. Surprisingly, however, about 10% of women with TNBC — those with the LAR subtype — had tumours overexpressing the androgen receptor, suggesting that these cancers are driven by hormones that are not usually associated with breast cancer. Pietenpol suspects that androgen-receptor antagonists used for treating prostate cancer might be commandeered for treating this TNBC subset. "It's a wonderful example of how subtyping a disease at the bench gives pretty quick insights as to how you could benefit patients," she says.

Pietenpol's group also showed that TNBC cell lines modelling these six subtypes responded to drugs that were selected according to the gene-expression profile. For example, cells representing BL1 and BL2 subtypes are predicted to have defective DNA repair and be genomically unstable. These cells were most sensitive to cisplatin, a drug that induces DNA lesions and death in cells that can't repair DNA damage.

The future options for TNBC therapy are widening. Clinical trials blocking other molecular targets are under way. Carey is involved in a trial of cetuximab, which inhibits the epidermal-growth-factor receptor (EGFR), a molecular marker of basal-like tumours. So far, the gains with cetuximab have been modest, as tumours seem to be finding ways to escape EGFR inhibition. Still, she's not giving up on this approach. "These are among the most genetically unstable tumours there are. Their ability to circumvent [treatments] is not that surprising."

And Pietenpol is keen to move discoveries to the clinic as quickly as possible. Patient numbers dictate that the focus is on finding treatments for patients with basal-like breast cancer, but she is confident about opportunities for the much smaller LAR patient group. "Any progress made for any of the subtypes will be significant; it's no different from Glivec," she says, referring to imatinib, the kinase-targeted drug that is highly effective against several cancer subtypes. "If you have a very good outcome based on a more precise therapy, then that can change the face of oncology care for patients. And that's what we're in this for." ■

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