Meeting report

A rational approach to cancer therapy

Kylie L Gorringe* and Ian G Campbell*†

Addresses: *VBCRC Cancer Genetics Laboratory, Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia. †Department of Pathology, University of Melbourne, Melbourne, Victoria 8006, Australia.

Correspondence: Ian G Campbell. Email: ian.campbell@petermac.org

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A report on the 20th Annual Lorne Cancer Conference, Lorne, Australia, 14-16 February 2008.

Targeted molecular therapy for cancer is becoming a holy grail for researchers. The identification and characterization of cancer genes is the first step towards developing such therapeutics, and high-throughput technologies are increasingly being used to identify these genes, elucidate their function and identify potential drug molecules that target them. At all levels of this process, a deep understanding of the molecular pathways involved is crucial to the successful development of a new therapeutic. In particular, combinations of therapies that each target aspects of the same or interacting pathways offer possibilities for synergy, reducing overall drug exposure and side effects for the patient. These themes were well covered at this years's Lorne cancer conference.

High-throughput technologies

The impact of high-throughput technologies on cancer research was electrifyingly demonstrated by Mike Stratton (Wellcome Trust Sanger Centre, Cambridge, UK), who showed how next generation massively parallel sequencing technologies can be used to determine the fine structure of chromosomal rearrangements. He described published work on the identification of rearrangement breakpoints in cancer cells. This involved the sequencing of large numbers of bacterial artificial chromosome clones with mismatched end sequences, each clone representing an individual rearrangement. The incredible complexity of these rearrangements could not have been appreciated without the level of detail that deep sequencing can provide, and led Stratton to describe a new model for the life history of these "deranged architectures" containing "genomic shards". He proposed that these latter small sequences, which range from 60 bp to a few kilobases, could arise through degradation of double-strand breaks (DSBs) and that they are captured by the repair machinery in an attempt to heal other DSBs, primarily through non-homologous end joining. This model is in contrast to the 'breakage-fusion-bridge' cycle previously proposed for gene amplification. Stratton also described some new work using the Genome Analyzer® system from Illumina, which enables not only short sequence reads but also the measurement of copy number based on the representation of each sequence within the population. The copy-number output Stratton presented exceeded even the level of resolution obtainable by the Affymetrix SNP 6.0 array, which with over 1.8 million probes is currently the leader in high-resolution copy-number mapping. The two outputs - sequence and copy number - could then be combined to look at the structure of gene amplicons, for example, to identify fusion genes.

An alternative method of identifying cancer genes was described by Anton Berns (Netherlands Cancer Institute, Amsterdam, the Netherlands). In this approach, integration of viral sequences into the mouse genome initiated tumor growth, leading to identification of the gene responsible through transposon tagging of the insertion site. His group has characterized more than 1,000 mouse lymphomas by sequencing each of the 20 insertion sites per tumor, more than half of which lay within genes. Interestingly, the frequency of detection of a particular insertion site depended on the genetic background of the mouse, which helped link the identified gene to a biochemical pathway. For example, tumors arising in a *p53*-knockout background were more likely to have insertions in the gene for cyclin D3, *Ccnd3*, than were tumors on a wild-type background. Of the common genes identified in the screen, only 15% overlapped with known human cancer genes such as the retinoblastoma gene *RB1*, suggesting that the remainder might represent novel targets of amplifications and deletions.

Characterizing genes and pathway interactions is clearly a crucial step in elucidating oncogenesis, and high-throughput analysis of pathways in model organisms offers one way of doing this. Norbert Perrimon (Harvard Medical School, Boston, USA) described a remarkable resource for high-throughput screens in *Drosophila*. The Drosophila RNAi Screening Center (DRSC) is building libraries of RNAi and cDNA clones to ultimately cover the entire *Drosophila* genome. More than 70 screens using this resource have been carried out, 28 of which have been published. Perrimon described screens using impressive confocal microsopic readouts with semi-automated fluorescent methods for detecting and scoring morphological features of the cells under study in order to identify genes and pathways that control cell shape, for example.

Understanding of pathways leading to targeted and combination therapies

How biochemical pathways can be used to find new therapeutics was illustrated by David Lane (University of Dundee, UK), who described a screen aimed at identifying small molecules that would activate p53. Tumors with wild-type *p53* but perturbation of the pathway through inactivation of *CDKN2A* inactivation or overexpression of *MDM2* would be specifically targeted by these drugs to reactivate the p53 pathway. Like nutlin (a *cis*-imidazole), these drugs sensitize the tumors to the effects of other therapies in combination, while reducing side effects on normal cells. The screen, encompassing 34,000 different molecules, utilized a reporter construct to detect p53 transcription factor activity. The 33 reproducible 'hits' were then used in a yeast genetic screen to identify the target genes. The small-molecule screen thus not only found new potential therapeutic agents, but also new p53 pathway genes, encoding the sirtuins, which function as deacetylases. Lane also described screens using small concentrations of each drug to identify those combinations that provide synergism, thus increasing selectivity and potency as well as reducing genotoxicity and side effects.

Drug combinations, this time targeting the apoptotic pathway, were the focus of a talk by Suzanne Cory (Walter and Eliza Hall Institute, Melbourne, Australia). The chemotherapeutic cyclophosphamide acts through apoptotic pathways that tumors may often be resistant to. Cory described how when combined with cyclophosphamide, the BH3 mimetic ABT-737 has been shown to increase survival and decrease side effects in a mouse model compared to cyclophosphamide alone. Similarly, Mike Bishop (University of California, San Francisco, USA) who gave this year's Ashley Dunn Oration, described several mouse models in which drug combinations and targeted molecular therapies result in improved survival. His optimistic and warmly received talk covered the targeting of several oncogenes, including *MYC* and the *PML-RAR* fusion gene. He described the drug VX-680, which kills only MYC-expressing cells through a combination of apoptosis and delayed toxicity via autophagy. Interestingly, autophagy continued to occur even when the drug was removed after the initial treatment, thereby decreasing side effects.

One of the looming unresolved issues in targeted therapeutics is the evolution of drug resistance. In a stand-out talk, Alan Ashworth (Breakthrough Breast Cancer Research Centre, London, UK) described the rational identification of PARP inhibitors (PIs) that specifically target tumors with defective DNA repair pathways, such as *BRCA1*- and *BRCA2*-deficient tumours. This class of compounds was identified through a synthetic lethality screen, in which the cell, already sensitized to DNA damage by loss of *BRCA1/2*, is overwhelmed by the combination of a chemotherapeutic agent that causes DNA damage and a compound that prevents DNA repair through an alternative pathway, in this case base excision repair. This combination results in the cells sustaining fatal genomic instability. In elegant work that has recently been published, Ashworth's group proactively looked for mechanisms of PI resistance in the CAPAN-1 (*BRCA2* null) cell line by treating the cells with the drug and identifying the genetic alterations in resistant clones. Fascinatingly, the resistance was found to be mediated by deletions within *BRCA2* that removed the original germline frameshift mutation, restoring the coding sequence to the correct frame with an in-frame fusion that, while still missing parts of the protein, appears to have sufficient function to restore DNA repair and enable resistance. Similar deletions were identified in ovarian cancer patients with resistance to carboplatin. Ashworth also addressed a question that frequently arises when discussing resistance to therapies - whether resistance arises through a conventional 'Darwinian' selective mechanism or by some type of *de novo* response to the presence of the drug. Ashworth supported the more logical Darwinian mechanism, by which resistance existing in a small proportion of tumor cells gives them a selective advantage upon treatment, allowing the clone containing the mutation to expand. At present there is no known mechanism for a resistance-enabling mutation to arise *de novo* in response to drug treatment. The Darwinian mechanism still remains to be tested in the context of drug resistance in cancer cells, but the high-throughput technologies now available could provide the means for a definitive experiment.

Although the development of targeted therapies has been slow so far, their promise is incontrovertible. Intelligent use of the high-throughput tools now available is accelerating the pace of discovery such that rational drug design will become the rule, rather than the exception.

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