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Converting cancer mutations into therapeutic opportunities

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While the use of synthetic lethality has been around for decades in model organism studies, it has only recently been applied to cancer therapy and judging by recent results, with great success. Following on a recent paper that demonstrates the clinical application of this strategy (Fong et al, 2009), Chris Lord and Alan Ashworth further explore the approach and show that poly(ADP-ribose) polymerase (PARP) inhibitors such as Olaparib can selectively target cancer cells defective in phosphatase and tensin homologue (PTEN, Mendes-Pereira et al, 2009) and that methotrexate is selectively lethal to MutS-homologue-2 (MSH2)-deficient tumour cells (Martin et al, 2009).

Cancer generally results from the accumulation of genetic alterations endowing cells with properties that result in unrestrained growth, proliferation and survival. Such genetic alterations appear in many flavours, such as point mutations that cause the affected genes to increase or decrease activity, gains or deletions in chromosomal regions causing increased or decreased expression of the affected genes, as well as chromosomal fusion events that generate entirely new chimeric proteins with altered functions. In most cases, these genetic alterations are benign, or so deleterious that the lesion promotes the removal of the affected cell from the population. In rare cases, however, such genetic alterations can cause cells to proliferate faster, or survive inappropriately *via* the activation of oncogenes, or inactivation of tumour suppressors.

An example of such a rare case is the deletion and/or mutation of phosphatase and tensin homologue (PTEN), deleted on chromosome 10. PTEN is a classic example of a tumour suppressor. The gene was discovered in 1997 as the relevant locus on a region of chromosome 10 that is deleted in multiple advanced cancers. Moreover, the remaining allele in these tumours was found to be frequently mutated, thereby conforming to the conventional two hit Knudson model of a tumour suppressor. A seminal observation from Jack Dixon's group showed that the protein encoded by this gene was a phosphatase that dephosphorylated the 3′ phosphate on the inositol ring of phosphatidyl inositol 3,4,5 tris phosphate (PIP3), a phospholipid known to be important in many aspects of cell growth and survival, providing a mechanism to account for its tumour suppressive properties (Maehama & Dixon, 1998). Other genes frequently mutated in sporadic tumours, are components of the DNA damage repair pathway, with some of the most well characterized examples including mutations in breast cancer (BRCA) or the Fanconi-anaemia (FA) complex, both of which are involved in homologous repair (HR). Patients who inherit BRCA1 or BRCA2 mutations, have an increased susceptibility to develop breast and ovarian cancers, whereas FA patients can have congenital defects and are predisposed to developing leukaemia and various solid tumours. A key factor underlying this tumour susceptibility is presumably the increased genetic instability of these cells due to the lack of an appropriate DNA damage surveillance mechanism.

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Once the genetic abnormalities in cancer cells have been documented, the tricky part is how to use this information to design effective therapeutics. A logical strategy is to inhibit the function of activated oncogenes—this works well when the oncogene is 'druggable' in some way, and good examples of this strategy include the antibody herceptin that inhibits the function of amplified human epidermal growth factor receptor 2 (HER2), and the small molecule imatinib that inhibits the activity of the chimeric Bcr-Abl kinase present in chronic myelogenous leukaemia. An alternative approach, which is effective even when the genetic alteration is difficult to reverse, is to isolate a drug that only kills cancer cells which harbour a particular genetic alteration, or which are compromised by

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other cancer-specific events. Such a concept is termed synthetic lethality, and has been a mainstay of yeast genetics for decades. While the mutation of any two individual genes is not lethal, combined mutations in both genes causes cell death. This approach can identify pathways, complexes and functional redundancies that would be difficult to elucidate by conventional means.

A recent example of the application of synthetic lethality was demonstrated in the context of breast cancer cells lacking BRCA1 or BRCA2. It was elegantly shown by two groups in 2005, that cells containing mutations in BRCA1 or BRCA2 are extremely dependent upon the activity of Poly(ADP-ribose) polymerase (PARP1), an NAD^+ utilizing enzyme that is involved in base excision repair (Bryant et al, 2005) (Farmer et al, 2005). A small molecule PARP inhibitor was 100–1000 times more effective in killing cells lacking BRCA1 or BRCA2, compared to isogenic wild type (WT) cells. This high degree of selectivity is attributed to the fact that cells deficient in HR, rely upon PARP, to repair ongoing single stand breaks that arise during growth; however, when PARP is inhibited, the single strand breaks degrade into double strand breaks that cannot be repaired due to the defect in HR. A follow up publication by Ashworth and coworkers, further solidified the connection between components of the HR pathway and reliance on PARP dependency, as a synthetic lethal short interfering RNA (siRNA) screen showed that cells deficient in numerous components of the HR pathway (*e.g.* RADiation sensitive mutants initially identified in yeast (RAD51), Fanconi anemia, complementation group D2 (FANCD2)) were extremely sensitive to killing by a PARP inhibitor (McCabe et al, 2006).

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This observation leads to the concept of developing a PARP inhibitor as an agent that specifically targets tumours deficient in BRCA1 or BRCA2 (mutations typically found in breast and ovarian cancers). Since the initial demonstration of this specificity, numerous PARP inhibitors have entered clinical trials (including AZD-2281 (Olaparib, Astra Zeneca), ABT-888 (Abbott), BSI-201 (BiPar sciences), MK-4827 (Merck) and AG-14699 (Pfizer)). In many cases, PARP inhibitors are being developed in combination regimens, as inhibition of PARP can potentiate the effects of numerous DNA damaging agents (*e.g.* combinations with temozolomide, irinotecan), but clearly a more targeted approach is to develop a PARP inhibitor in the context of patients with defects in BRCA. A recent Phase 1 clinical trial with the PARP inhibitor AZD-2281 (Olaparib) has dramatically reproduced this preclinical concept of synthetic lethality. As reported by Fong et al (Fong et al, 2009), of 60 patients administered with increasing doses of AZD-2281, only BRCA carriers (12/19) showed clinical benefit from AZD-2281, and did so with minimal side effects. While this appears to validate the use of AZD-2281 in BRCA mutant tumours, the authors point out that there may be additional tumours that also show defects in homologous recombination repair that could benefit from this treatment.

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As reported in this issue of EMBO Molecular Medicine, a surprising group of additional tumours that might benefit from PARP inhibitors, are those harbouring PTEN mutations (Mendes-Pereira et al, 2009). While the role of PTEN in antagonizing phosphoinositide 3 (PI3)-kinase activity is well documented, a more recently described role for PTEN is in the maintenance of chromosomal integrity. Ramon Parsons and coworkers reported that PTEN null cells showed a defective checkpoint in response to ionizing radiation, resulting in unrepaired chromosomes and aneuploidy (Puc et al, 2005). Similarly, Pandolfi's laboratory confirmed extensive chromosomal instability in PTEN null cells due to decreased levels of RAD51, an important component of double strand break repair (Shen et al, 2007). Following on from these observations, Mendes-Pereira et al in this issue of EMBO Molecular Medicine, show that cancer cells lacking PTEN have decreased levels of RAD51, and a corresponding decrease in HR-mediated DNA repair. Given this finding, it was not surprising that PTEN-deficient cells are 20-fold more sensitive to PARP inhibitors, an effect that was reversed upon expression of PTEN or RAD51. Interestingly, the effect of PTEN in this process did not depend on its phosphatase activity, but was at least partially dependent on its ability to shuttle to the nucleus. Previous nuclear, non-phosphatase-dependent effects of PTEN have been noted, including binding and activation of p53, and binding and inactivation of the nuclear proto-oncogene microspherule protein 58 (MSP58). It will be interesting to elucidate the mechanism underlying PTEN's function in this process.

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In another separate paper in this issue, in trying to identify other agents that are tumour type specific, Ashworth and coworkers went on to screen a focused small molecule library to identify a compound that selectively targets one particular mutated cell type (Martin et al, 2009). They utilized a library of 1120 compounds, 90% of which have been clinically approved, to screen human endometrial carcinoma cells 59 (Hec59) cells which are deficient in MutS homologue-2 (MSH2), one other component of the DNA mismatch repair pathway (MMR). Defects in the MMR pathway are frequently found in hereditary non-polyposis colorectal cancer. To identify compounds that selectively kill MMR^- cells *versus* MMR^+ genetically matched cells, they used cells in which Chromo-

some 2 (which contains wild-type MSH2) had been transferred into the MSH2 negative Hec59 and compared the sensitivity of both (Martin et al, 2009). This resulted in the identification of a drug that is selective for MMR deficient tumours and could have utility in this patient population. Methotrexate, a drug first used clinically 60 years ago by Sidney Farber, was identified in this screen and found to reduce viability of MSH2 deficient cells relative to MSH2 wild-type cells (by >140 fold). Methotrexate inhibits dihydrofolate reductase (DHFR) and it was shown that inhibition of DHFR results in an increase of free radicals that induce 8-hydroxydeoxyguanosine (8-OHdG) lesions in DNA; cells deficient for MSH2 have a reduced capacity to repair these lesions. Importantly, the authors also demonstrate that methotrexate treatment reduced *in vivo* tumour growth of MSH2-deficient Hec59 cells specifically. It is surprising that MutL homologue 1 (MLH1) deficiency, also a component of the MMR pathway, does not elicit the same response to methotrexate. Additionally, as MSH2 form the MutS a lesion recognition complex, it would be very interesting to test whether MSH6 is required for methotrexate resistance. Nevertheless, the key to a successful clinical trial with methotrexate is the challenge of correctly identifying and enrolling patients who are only deficient in MSH2 (and pre-determining what level of functional inactivation defines 'deficiency'). Even though this is likely to be a small population, it is a great example of targeting a population that can benefit most from a specific drug.

One of the most attractive aspects of the use of synthetic lethality in the context of human tumours is that, it can be used against targets that have thus far proven very difficult to drug directly. For example, mutations in the small guanosine triphosphate GTPase Ras are found in 30% of all human tumours, yet attempts to inhibit Rat sarcoma (Ras) have so far been unsuccessful. Using small molecule libraries on pairs of cell lines that are identical apart from the presence or absence of mutant Ras, compounds and genes have been isolated that appear to be selective for mutant Ras expressing cells (reviewed in Sawyers, 2009). A similar approach identified cyclin-dependent kinase 6 (Cdk6) as being required for cells lacking the tumour suppressor (Von Hippel-Lindau (VHL)) (Bommi-Reddy et al, 2008). In all cases these studies isolated more conventionally 'druggable' targets for tumours with these genetic lesions, and indeed, Cdk inhibitors were shown to be effective in killing VHL mutant cell lines.

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Clearly, one of the key goals of any therapeutic is to use it within the patient population that is most likely to benefit from its use. There are many examples of clinical trials that have elicited a poor initial response, yet once the data was analysed in the context of distinct subpopulations, it became clear that a certain genetic profile responded better than the overall population ((*e.g.* herceptin and HER2 positive breast cancer, and recently epidermal growth factor receptor (EGFR) inhibitors for non-small cell lung carcinoma (NSCLC)). Significant effort could have been saved if it was known ahead of time, which population was more likely to respond. The studies discussed here are very clear examples of identifying a simple genetic test to stratify patients who are more likely to respond to a particular drug. Hopefully, this early positive clinical data with AZD-2281, will encourage further development of this approach to additional patient populations and drugs.

The authors declare that they have no conflict of interest.

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