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synonymous with 'biology'—biology against rich tableaux of sequence information. Who does not remember the seminars in which the speaker professed an interest in some biological phenomenon, and then nose-dived into a 40-minute description of gene mapping, cloning and sequencing? With the completion of the genome projects, one senses that these days will soon be over: back to biology. □

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A molecular blueprint for targeting cancer?

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Wouldn't it be fabulous if inspection of a tumour would immediately suggest the most effective therapy for total eradication of the disease? The ability to rapidly translate the defects of a tumour cell into a molecular blueprint for therapeutic targeting is an important step in the realization of this dream. To this end, researchers have charted every conceivable quality of tumour cells—including biochemical peculiarities and genetic abnormalities—with the hope of identifying signature changes that indicate the critical defects of a given tumour's physiology. Armed with this accumulated knowledge, we may soon be able to customize therapy. For this approach to be effective, we need techniques that can rapidly scan the total genome for mutations, and an understanding of the functional significance of the mutational spectra. On page 99 of this issue, Laleh Shayesteh and colleagues provide a nascent example of the latter; they present data suggesting that *PIK3CA*, encoding PI3-kinase, is an oncogene important in ovarian cancer by virtue of its frequent amplification in these tumours.

Karyotype analysis has, for some decades now, provided a fruitful approach to characterizing tumour cells. The molecular characterization of disease-specific, non-random mutations has allowed the identification of critical tumour suppressor genes through the analysis of deletions, and analyses of translocations and amplifications have implicated oncogenes. The chromosomal abnormalities that result from amplification of a genetic locus, the intrachromosomal homogeneously staining regions (HSRs) and the extrachromosomal double minute chromosomes (DMs), have long held the promise of providing clues to the functional defects in tumour cells (Fig. 1). June Beidler first suggested that these chromosomal anomalies, associated with methotrexate (MTX) resistance, may harbour an increased number of genes that confer drug resistance[1]. The discovery of dihydrofolate reductase gene amplification[2] subsequently confirmed this hypothesis—and was soon followed by numerous other examples.

For defined phenotypes such as drug resistance, identification of the amplified gene is straightforward because manipulation of the candidate gene may verify or refute its effect. Furthermore, the selection pressure—in this case, growth in the presence of drug—is defined. For more complex phenotypes, such as tumorigenic potential, the complexity of the variables involved in generating the phenotype renders analysis immensely more difficult. For this reason, most genes that respond to selection pressure and drive the plethora of amplifications and deletions identified by chromosomal analysis of tumour samples are currently unknown.

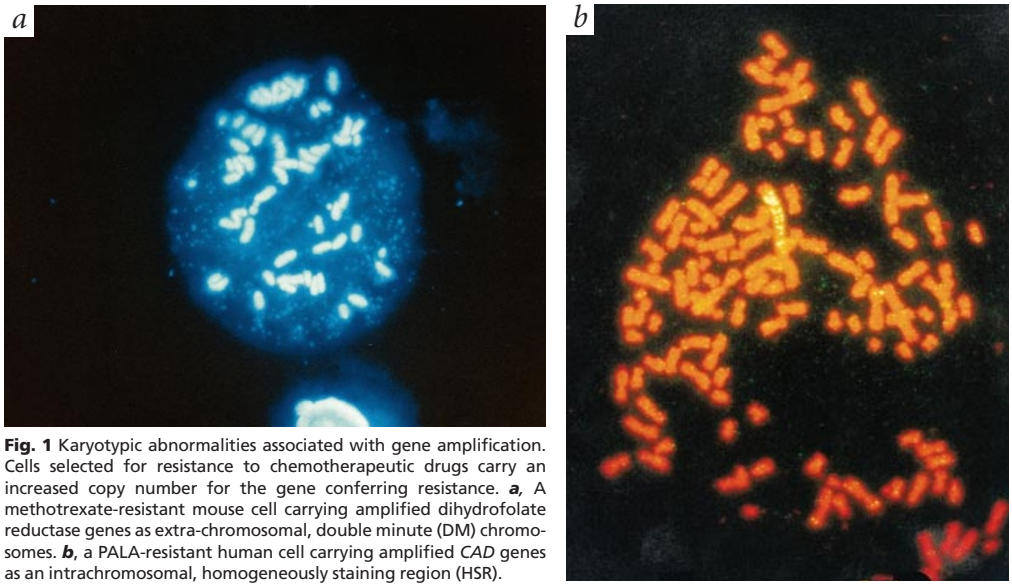

Fig. 1 Karyotypic abnormalities associated with gene amplification. Cells selected for resistance to chemotherapeutic drugs carry an increased copy number for the gene conferring resistance. ***a,*** A methotrexate-resistant mouse cell carrying amplified dihydrofolate reductase genes as extra-chromosomal, double minute (DM) chromosomes. ***b***, a PALA-resistant human cell carrying amplified *CAD* genes as an intrachromosomal, homogeneously staining region (HSR).

Comparative genomic hybridization (CGH) is a powerful cytogenetic tool for identifying changes in gene copy number within the entire genome and has substantially improved our capacity to identify mutational changes of individual tumours, and loci that are specifically altered in a tissue-specific fashion. Shayesteh *et al.* used CGH to specify an amplicon present in ovarian, lung, head and neck, urinary tract and cervical cancers. In search of the gene whose selection drives its amplifi-

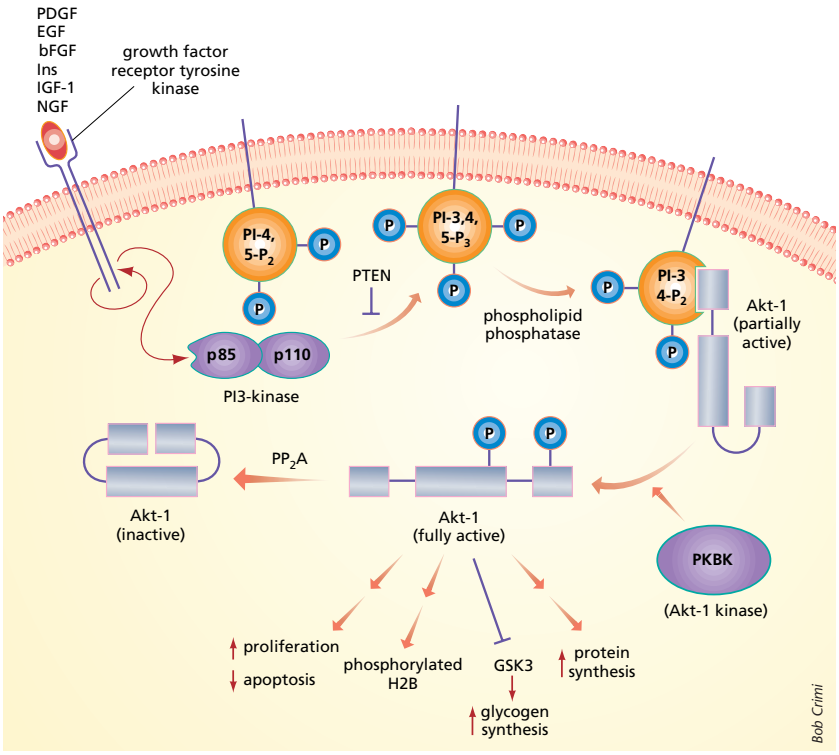

Fig. 2 Recruitment and activation of PI3-kinase. Activation of growth factor receptors triggers the activation of PI3-kinase by phosphorylation. The PI3-kinase phosphorylates membrane lipids which, after subsequent modification, produce the second messengers PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3. As a result, Akt-1 is recruited to the cellular membrane, where its phosphorylation alters its conformation and activates its kinase domain. Phosphorylation of downstream targets by Akt-1 effects multiple cellular processes. The tumour suppressor gene, *PTEN*, encodes a phosphatase that antagonizes the activation of the second messengers. Akt-1 activity results in the phosphorylation of substrates that mediate events in carcinogenesis.

cation, the authors found *PIK3CA*, a gene encoding the p110α catalytic subunit of phosphatidylinositol 3-kinase (PI3-kinase), to be an attractive candidate.

Akting up

p110α couples with an adaptor protein, p85, to generate a kinase activity capable of mediating signals for a wide variety of cellular functions implicated in tumour formation and progression (Fig. 2). These include cell proliferation, suppression of apoptosis, glucose transport and catabolism, cell adhesion, vesicle transport and endocytosis, which are also effected by an oncogene, *AKT1* (refs 3,4). Extensive evidence supports the view that PI3-kinase is recruited and activated by a variety of growth factor receptor tyrosine kinases to generate second messengers by phosphorylating inositol lipids at the D3 position[5,6]. The consequent dephosphorylation of PI3-kinase results in derivatives (phosphatidylinositol-3,4-bisphosphate (PtdIns-3,4-P_2) and PtdIns-3,4,5-P_3) that permit the binding and activation of the serine/threonine protein kinase, Akt-1 (also called PKB; ref. 7). The activation of Akt-1 may be inhibited by either physiological means (such as the expression of *PTEN*; refs 8,9) or pharmaceutical means (such as exposure to wortmannin or LY294002).

This knowledge should now permit a functional analysis of the amplification event in ovarian cancer. If the candidate gene is responsible for the proliferative advantage enjoyed by the cells bearing the amplified segment, it would be expected that: (i) the gene sits within the amplified region; (ii) expression of the gene product is commensurately increased; and (iii) its functional activity is increased. With respect to *PIK3CA*, all three predictions are met, firmly establishing a correlation consistent with the idea that PI3-kinase over-expression plays a role in ovarian tumor biology.

Definitive demonstration that a candidate gene is indeed responsible for a given phenotype rests with the manipulation of a gene product within its functional epistatic pathway and a concomitant, predicted effect on phenotype. For example, if over-expression of a locus is important in carcinogenesis, then over-expression of that locus in non-tumorigenic cells may be predicted to transform them. Conversely, inhibition of the same activity by genetic or pharmacological means may be expected to reverse some aspect of tumorigenicity. Accordingly, Shayesteh *et al.* noted a diminished-growth response to LY294002 by ovarian cell lines containing amplified *PIK3CA*. Identifying in a definitive manner which of the multiple physiological effects that are mediated by PI3-kinase is responsible for tumorigenesis or tumour progression is a tall order. In general, this is especially true if the candidate gene product acts late in carcinogenic progression, when its action may depend on the mutational context of the tumour cell, which resides within an heterogeneous, constantly-changing population.

In an alternative approach, additional analysis of the molecular blueprint of the cancer itself may provide evidence for the functional significance of certain mutational changes. Precedence for this has been seen with the inactivation of p53 protein function by high risk HPV16 E6 oncoprotein; cells that inactivate p53 by E6-mediated degradation of the protein usually do not contain mutations in *TP53*. An even more germane example is the elimination of *DHFR* amplification upon acquisition of a secondary mutation in the *DHFR* coding sequence that decreases the affinity of the protein for MTX. Relaxation of selection pressure by the point mutation obviates the need for the amplified copies[10]. By way of analogy, any mutation in *PIK3CA* that has the net effect of increasing Akt-1 activity should relax selection pressure for the amplification of *PIK3CA* if, indeed, increasing Akt-1 activity is the pivotal functional endpoint of the amplification. Additionally, one might predict that deletion of *PTEN* and amplification of *PIK3CA* are unlikely to occur in the same tumour cell, as each allows for increased PI3-kinase activity and activation of downstream effectors of Akt-1. The identification of signature chromosomal abnormalities and the elucidation of their functional significance is not merely an academic exercise. New therapeutic stategies can be developed which provide a focused attack on the unique cells carrying the aberration. The development of Herceptin as a therapy for mammary cancer cells containing amplified *HER2* is predicated on the success of this approach. Hopefully, other examples will soon follow. □

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