Shibboleths in the accents of cancer

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Dangerous words

"…said they unto him, Say now Shibboleth: and he said Sibboleth: for he could not frame to pronounce it right. Then they took him, and slew him…" Judges 12:6

Cancers remain relatively intractable diseases that continue to pose significant challenges for biomedical science. One reason for this is the paucity of cancer-specific molecular targets at which therapeutic drugs may be directed. Hence the side-effects usually associated with cancer for example, mucositis (ulceration of the alimentary canal, eg mouth and throat, causing difficulty in eating), or hair loss. In some cases, the cancer cells cannot be eradicated without unacceptable damage to normal tissues, and the tumour is effectively untreatable.

This situation has driven the relentless molecular dissection of cancers, in an effort to find targets which, if not entirely specific to cancer cells, at least may represent critical levers at which application of pressure may reverse the malignant phenotype to a non-progressing or even normal set of behaviours. One area in which cancer cells frequently differ from their normal counterparts is that of cell signalling, the complex process of communication between the cell nucleus and the cell's external environment. The hope is that the study of these differences will yield a therapeutic 'shibboleth' by which a drug could distinguish cancer cells from normal cells, allowing development of therapies that cause minimal side effects. In this article we briefly introduce the concept of cell signalling and examine the extent to which the study of cancer cell signalling may yield therapeutic targets.

It's good to talk

For cells, communication with the outside world is not optional, but essential for normal functioning. Environmental cues, such as small molecules (eg nitric oxide), circulating proteins (eg steroid hormones and cytokines), structural matrix proteins or membrane-bound proteins on adjacent cells, may be detected by the cell and result in a variety of behavioural changes. These changes may take the form of immediate reactions, as when neurons respond to neurotransmitters by membrane depolarisation. They may also take the form of rapid metabolic responses, as when pancreatic beta cells respond to blood sugar levels through secretion of insulin.

Slower and more sustained changes in cell behaviour also are evoked by signals in the environment, for example when cells respond to environmental cues by increasing their rate of proliferation. Such changes are due to alterations in gene expression, causing the cell to produce a different set of proteins, or to produce particular proteins in smaller or greater quantities. This in turn endows the cell with a different set of behaviours and abilities, including the ability to influence the behaviour of other cells through similar signalling mechanisms. It is these

sustained alterations in behaviour which are generally thought to be of most importance in the progression of cancer.

Shibboleth

A review of the molecular biology of cell communication is far beyond the scope of this article. Here we briefly overview the importance of a single group of signalling molecules, the tyrosine kinases, in cell signalling and cancer therapy.

Tyrosine kinases are enzymes which have the ability to add phosphate groups to tyrosine groups in proteins; this protein phosphorylation is a general mechanism for converting functional proteins from a dormant state to a reactive state, and is critical in the process of communicating cell surface binding events to the cell nucleus. The receptor tyrosine kinases comprise a subgroup within the family of tyrosine kinases, and are receptors (molecules with a specific affinity for another molecule) embedded in the cell membrane. Receptor tyrosine kinases have extracellular domains which bind specific molecules, and intracellular domains which are available for interactions inside the cell. A binding event in the extracellular region may cause a change in the intracellular tail of the receptor which then triggers a cascade of reactions within the cell, eventually resulting in changes in gene transcription.

Molecular signals that are recognised by the receptor tyrosine kinases include epidermal growth factor, platelet derived growth factor, and vascular endothelial growth factor, all of which promote cell proliferation and which have implications in cancer progression. Binding of a signal molecule to two cell surface receptors simultaneously results in the two receptors associating into an activated transmembrane complex. This complex has tyrosine kinase activity, ie can activate proteins by transfer of phosphate groups. Some of the proteins which are activated by the receptor complex are cytosolic tyrosine kinases which then proceed to activate other proteins in a cascade of reactions. Some activated proteins may enter the nucleus and transfer their phosphates to transcription factors, thereby activating them and allowing them to bind to DNA and promote transcription from specific genes.

Sibboleth

So much for normal signalling; but the question is, how do cancer cells communicate, and is the language of cancer sufficiently different from that of normal cells to comprise a source of drug targets? Below we give a brief overview of some of the better-known idiosyncracies of cancer in tyrosine kinase-regulated cell signalling.

According to our current state of knowledge, it seems that the tyrosine kinases may be particularly important elements in cancer progression. More than 70% of the known cancer-associated genes are said to encode tyrosine kinases. For example, two of the four known human epidermal growth factor receptors, the HER1 and HER2 receptor tyrosine kinases, have been shown to be associated with cancer when defective, and hence are targets for cancer therapy.

In some cases the malignant phenotype may be associated with quantitative changes in the receptor. Thus HER1 overexpression is found in many solid tumours, eg lung cancer, and HER2 is overexpressed in a significant percentage of breast cancers. In other cases the malignant phenotype may be associated with qualitative changes in the receptor. For example, some HER1 mutations (eg truncations of the protein, or amino acid changes that result in continuous association of pairs of receptors) permanently activate the receptor in the

absence of a signal or binding event. Other HER1 mutations may affect the way the HER1 signalling cascade interacts with signalling regulated by other receptors. (This 'crosstalk' between pathways may have various effects on proliferation, migration and other facets of tumour behaviour).

Apart from the HER receptors, other receptor tyrosine kinase genes that may be of interest in cancer progression include *Fms* (the CSF-1 receptor); *Trk* A, B and C (receptors for NGF-like proteins); *Flg* (receptor for FGF); *Kit* (mast cell growth factor receptor); and *Met* (hepatocyte growth factor receptor). In addition, *Src*, a gene commonly associated with cancers when mutated, is a membrane-associated tyrosine kinase that is not a receptor.

The abnormality in the signalling pathway may be due not to a defect in the tyrosine kinase itself but in the proteins which interact with it. For example, a mutant protein (BCR/ABL) associated with chronic myelogenous leukaemia continually phosphorylates a cytosolic receptor tyrosine kinase, resulting in overexpression of genes which stimulate cell proliferation. A similar autocrine loop is found in some cancer cells which secrete their own growth factors, such as epidermal growth factor (thereby causing essentially permanent activation of the HER receptor and resulting in uncontrolled proliferation). RAS and RAF are kinases which appear to be intermediaries in the pathway by which some receptor tyrosine kinases activate proliferation-associated genes, and mutated *Ras* and *Raf* genes are associated with cancers. It is said that 10-20% of all human tumours contain a mutated RAF protein.

By their words ye shall know them

It seems then that in some cases cancers may exhibit elements of the communication process that are sufficiently idiosyncratic to allow them to be usefully distinguished from normal cells. For example, mutations in receptors or cytosolic kinases, or overexpression of receptors, might permit therapies to be targeted to cancer cells, at least to some extent. Below we briefly outline some of the strategies and drugs that have been proposed based on tyrosine kinase-regulated cell signalling in cancer.

In broad terms, one can target either the external, cell surface components of the signalling process, or the internal, cytosolic components. In the former case large molecules such as proteins can be used. Thus, the extracellular part of the receptor can be targeted with antibodies which interfere with ligand binding and prevent receptor activation. For example, Herceptin (trastuzumab) is an antibody that blocks HER2, a receptor tyrosine kinase which is overexpressed in about 25% of breast cancers, and Erbitux (cetuximab) is an antibody that blocks HER1 and is sometimes used in the treatment of metastatic colorectal cancer. It may also be possible to effect tumour regression by attaching toxic agents to antibodies which are specific for the receptors in question.

An alternative approach is to target the cytosolic components of signalling, eg through the use of small molecules that can interact with the intracellular part of the receptor. This approach may prevent the phosphorylation of tyrosine kinases and hence block initiation of the phosphorylation cascade that can alter gene transcription in deleterious ways. Blocking the phosphorylation cascade at the intracellular site may be a more effective strategy than using antibodies to block the extracellular domain, since in some cases it can block the permanent activation resulting from some types of receptor mutation, which an external binding event would not affect.

Examples of cytosolic tyrosine kinase inhibitors include Tarceva (erlotinib) and Iressa (gefitinib), which are small molecules that appear to specifically inhibit HER1-regulated tyrosine kinases through binding to the intracellular domain of the receptor. Similarly, Gleevec (or Glivec; imatinib mesylate) binds to the BCR/ABL tyrosine kinase in chronic myelogenous leukaemia and prevents phosphate donation by ATP, thereby stopping constitutive BCR/ABL phosphorylation activity, which otherwise would result in abnormal behaviour including uncontrolled proliferation.

Investigational compounds that may inhibit both HER1 and HER2-regulated tyrosine kinases, and other compounds that may inhibit all tyrosine kinases regulated by the HER family, are said to be under development. For example, lapatinib ditosylate is a small molecule inhibitor that appears to block signalling from both HER1 and HER2.

The word on the street

The importance to the cell of communication systems is suggested by the significant proportion of the genome that is set aside for encoding signalling molecules and transducers. Nearly 100 different tyrosine kinases so far have been identified in the human genome, of which over half are receptor tyrosine kinases, and the total number of tyrosine kinases has been suggested to be in the region of 1000. The fundamental role of these molecules in regulating some of the processes of life is further reflected in their apparent importance in the progression and maintenance of the transformed phenotype. Hence the current focus on developing drugs that interfere with cell signalling, in particular tyrosine kinase-regulated signalling. It is hoped that some of these drugs may interfere with the molecular pathways that drive critical elements of cancer progression (eg cell proliferation) sufficiently to slow or halt the spread of disease and thus turn the cancer into a chronic, largely controllable disease.

However, this view of the potential of the new inhibitor therapies may be overly optimistic. Tyrosine kinases and receptor tyrosine kinases are present in normal cells where they have necessary and beneficial functions. Therefore treatments which interfere with these molecules may not be entirely specific for cancer cells, and therefore are likely to have side effects. Indeed the clinical data to date indicate that this is so, and some tyrosine kinase inhibitors in fact may be quite toxic. In general, however, it seems that the side-effects from signalling inhibitors may not be as severe as the side effects produced by 'classical' cytotoxic drugs.

In addition, most cancers consist of relatively heterogeneous populations of cells from which resistant clones can grow under therapeutic 'selection pressure' which kills off their counterparts. It is unlikely that cell signalling inhibitors will be immune to the development of resistant clones over time. Part of the rationale behind the development of lapatinib is that a drug which targets two signalling pathways simultaneously may be less prone to resistance evolution by the cancer. However, even if this were so, one would expect more side effects, since two signalling pathways will also be affected in normal cells. Nevertheless, so far the drug is said to be reasonably well tolerated.

Ideally, one would design inhibitors that are specific only for the mutant forms of tyrosine kinase associated with some cancers, and these types of drug would be expected to be relatively free of side effects, although the problem of clonal outgrowth of resistant mutants from the original tumour population still would remain. Interestingly, gefitinib may quite by chance turn out to be a mutation-specific drug. Recent work suggests that responses are more common among patients who have mutations in the intracellular domain of HER1, leading to

speculation that the mutations stabilise the interaction with gefitinib and make it more effective. Perhaps future strategies will require tumour HER1s to be analysed for mutations and the patients to be stratified for treatment with gefitinib or other drugs accordingly.

Unfortunately, although this approach is attractive in concept, in practise there may be economic barriers to its implementation. It has been suggested that drug companies would be unlikely to bear the cost of developing new drugs for all of the various patient sub-populations represented by the range of mutations present in a given cancer, particularly in view of the lower returns from these patient sub-populations (as compared with the market for 'one size fits all' classical cytotoxic approaches). This does however depend on the size of the subpopulation in question, which in some cases may be large enough for development of a commercially viable drug.

At present then, it seems that signalling in cancer may represent a source of drug targets, but that therapies based on these targets are unlikely to be free from problems that have dogged other approaches, such as specificity (lack of side effects) and long-term efficacy (avoidance of outgrowth of resistant clones). Nevertheless some of the new inhibitor therapies represent clear improvements on previously available treatments, and others perhaps will be found to be particularly effective when used as part of a drug cocktail. As is usual with the development of cancer therapies, progress is slow, and we are more likely to see a series of incremental improvements than discovery of a magic bullet.

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