IMPORTANCE OF MOLECULAR GENETICS OF SARCOMAS

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Abstract

Sarcomas represent a paradigm for rare cancers. Rare cancers have historically presented significant challenges, because traditionally clinical trials require significant numbers of patients to achieve adequate statistical power. In part, this was due toe the lack of efficacy of the treatments used for sarcomas, and in part to the sheer heterogeneity of subtypes within this uncommon group of cancers. It is arguable that recent developments in molecular genetics are transforming the outlook for patients with rare cancers. Since the sequence of the human genome was published in 2001, the landscape of cancer genetics has changed forever. A combination of the accelerating progress in genomic technologies, together with the raft of molecularly targeted therapeutic agents, is fundamentally altering the face of clinical trials in sarcomas. These developments in molecular genetics of sarcomas, and their current and future impact on clinical care will be reviewed. These changes throw out a new challenge to clinicians treating rare diseases, and those responsible for health care systems to avoid being rate-limiting in translating science into clinical benefit.

Sarcomas are rare cancers, which are increasingly treated by a handful of experts in remote ivory towers. There are so many sarcomas, each more individually rare than the last! Many of them have dreadful consequences, and nothing has really changed, has it? Moreover, what earthly use is molecular genetics to a clinician at the coalface? The litany of unintelligible acronyms that constitute many papers on the molecular genetics may be enough to turn even the boldest off an article - even in *Cancer Forum.*

Let me persuade you to read on. In return, I will provide a satellite level overview of the genetics of sarcoma, pointing out key areas of interest and putting our patients right at the heart of the matter. I promise to avoid jargon as much as I can; if I do weaken, it will be to make an important general point. I do not intend to delve into each of the 70+ subtypes of sarcoma, but to illustrate my messages with examples that will make my meaning clear.

The rarest diseases can have the most important consequences - nowhere is this more true than for the collection of diseases collectively called sarcomas. That the future of modern cancer care is fundamentally changing, so fast that it is bewildering. However, it is increasingly critical for our patients that we bring the emerging world of molecular cancer genetics into our consulting rooms.

Molecular genetics, histopathology, and cancer classification

Do rare cancers like sarcomas really matter? With limited resources, utilitarians teach us that the greatest good for the greatest number should guide difficult decisions. It is arguable that we should place our money not only where the need is, but where it can make a difference. However, the question is cruel. No patient has ever been cheered by the thought that their cancer is rare; to the patient, rare cancers are life itself. But rare cancers do matter to all of us, because one in five cancers lies outside the top 10 (bowel, breast, prostate, lung and so on), and they cause one in three cancer deaths.[1] Rare cancers also have been traditionally neglected, because up until the past decade, we haven't really known how most of our treatments work. If you use a blunderbuss, the details don't matter. 'Treatment' or 'therapy' - in this context I mean drug treatments, unless otherwise stated.

A recent survey showed that there was an inverse relationship between the incidence of a cancer and the likelihood of approval of new drugs,[2] drugs that have fundamentally changed the outlook for affected individuals. Why is this so? It is because of something called genotype-phenotype relations. I am using this term (genotype-phenotype relations) to mean that a given pattern of genetic changes yields a defined, identifiable appearance. This concept is important, because it seems that the new treatments are mostly based on the underlying genotype, regardless of the appearance. Let me explain how this works, and how it applies to sarcomas.

It is increasingly accepted that the common cancers are genetically heterogeneous. For example, the entity formerly known as 'breast cancer' is rapidly evolving under the influence of molecular genetics into more than half a dozen different subtypes of cancer, according to oestrogen and progesterone receptor status, HER2 status, luminal A, luminal B, BRCA1/2 mutant, and so on. Some of these distinctions already have clinical significance. This situation will likely get more complex with time. Sequencing of cancer genomes reveals that there may be about 50 different mutations in each cancer, and that many of these mutations occur in less than 5% of what we previously considered one disease.[3] Thus, there is a poor genotype-phenotype relationship in many

common cancers. This is the reason that the epidermal growth factor receptor (EGFR) inhibitors failed to work in more than 10% of lung cancers, until we realised that we needed to target the underlying genotype (EGFR mutation positive lung cancer), after which our response rates increased dramatically.[4] In summary, where the phenotype does not reflect the underlying genotype, genotype is increasingly likely to trump phenotype in clinical importance.

For sarcomas, by contrast, genotype-phenotype relations are often (but not always) much more reliable. Sarcomas may be divided into several broad groups - those with defined molecular genetic abnormalities and those that more closely resemble the common epithelial cancers.[5] The latter (leiomyosarcoma, malignant fibrous histiocytoma (MFH), pleomorphic liposarcoma, osteosarcoma and so on) lack a characteristic genetic change. I will come to these later. The group of connective tissue tumours with defined molecular genetic abnormalities is increasing in number. It includes Ewing sarcoma, gastrointestinal stromal tumours (GIST), dermatofibrosarcoma protuberans, well/dedifferentiated liposarcoma, myxoid liposarcoma, pigmented villonodular synovitis and many more. These tumours have quite distinct appearances under the light microscope. This means that, for these diseases, the light microscope appearance of the cancer is predictive of an underlying genetic defect, and therefore may be used to guide treatment in many cases.

Know thy enemy

The impact of molecular genetics on the classification of sarcomas cannot be overstated. In the early 1990s, the misclassification rate in sarcomas based on histopathology (phenotype) was formally shown to be 15-20%.[5] This remains true today, with potentially devastating consequences. The problem is that sarcomas are rare and the subtypes are rarer, the clinical implications are not always immediately obvious, and molecular pathology is still not routinely available to back up the diagnosis.

For the so-called 'pleomorphic' sarcomas (leiomyosarcoma, MFH and so on), there is generally no clinically effective, targeted therapy (leaving out for a moment osteosarcoma). One consequence of this has been the creation (and imminent demise) of an entire category of sarcomas - MFH. The category of MFH (sometimes known as pleomorphic sarcoma, not otherwise specified) was created to allow pathologists to classify sarcomas without an obvious line of differentiation. Struggling to make out the line of differentiation did not appear to matter - there is still no underlying genotype identified and our chemotherapy treatments still do not really work well. A fine pathologist (Chris Fletcher, in Boston) showed that it is possible to reclassify MFH in almost 70% of cases,[6] and it is likely that MFH will be dropped as an entity from the next edition of the World Health Organisation atlas on the Pathology and Genetics of tumours of soft tissue and bone. The distinctions may not impact upon clinical care immediately, but there is every chance they will matter soon.

The distinction between leiomyosarcoma and MFH may not be critical (yet), but this is not the case for the distinction between synovial sarcoma (carrying a translocation between chromosomes X and 18) and Ewing sarcoma (translocation between chromosomes 11 and 22).[5] Although our treatments are not targeted (blunderbuss), they seem for unknown reasons to be particularly effective in these cancer types. In the case of Ewing sarcoma, an intensive and prolonged course of chemotherapy, combined with surgery and perhaps radiotherapy, is critical to cure. The tests we use to diagnose these cancers are based on the light microscope and cytogenetics. The appearance under the light microscope is fallible, as reported in the literature. The consequences of a misdiagnosis of Ewing sarcoma are very great for our patients. If we wrongly call a tumour 'Ewing sarcoma', the patient will receive nine months of intense chemotherapy, itself carrying a significant risk of mortality. If we wrongly fail to diagnose this cancer, the patient may not receive potentially curative treatment. Yet there is no government rebate for the cytogenetic test required to demonstrate the Ewing translocation, which is technically relatively simple and commercially available. Nowhere is a clinical need for molecular pathology more important than among the 70 or more diseases called sarcoma.

The clinical development of targeted therapeutics makes the need for good molecular pathology even more pressing, a point strikingly illustrated by GIST. Gut leiomyosarcoma and GIST were routinely conflated in the early 1990s, and GIST was thought to be relatively rare. These cancers were collectively unresponsive to drug treatments. In 1998, a Japanese group showed that GIST was due to mutations in the KIT gene, which encodes a growth receptor on the surface of that mysterious entity, the interstitial cell of Cajal.[7] In parallel with this, Novartis was developing a targeted drug (imatinib), whose targets include the platelet-derived growth factor receptor (PDGFR), the ABL kinase and the colony stimulating factor 1 receptor (CSF1R). When it became apparent that imatinib had a dramatic effect on GIST through its inhibition of KIT, not only did centres accessing the drug through clinical trials rapidly become inundated by patients, but the incidence of GIST mysteriously rose. A recent survey in France has shown that GIST is one of the single most common subtypes of soft tissue sarcomas.[8] It is now considered routine standard of care to test for mutations in KIT, in part because it is clear that different mutations respond differently to treatment.[9] The discovery of mutations affecting other imatinib targets has expanded the therapeutic applications of imatinib to dermatofibrosarcoma protuberans and possibly other connective tissue tumours.

Changing times: clinical trials as standard of care

An important theme is that the time from target discovery to proof-of-principle in cancer care is accelerating.[7,10] One implication is that the clinical classification of cancers needs increasingly to take into account the underlying genotype, for consideration of access to therapeutic

trials of novel agents as they become available. The standard of care is shifting rapidly, and the clinician is right at the centre of this trend. This is true for perhaps the most common soft tissue sarcoma, well-dedifferentiated liposarcoma. This disease is characterised by the near obligate amplification of two oncogenes, MDM2 and CDK4. While well-differentiated liposarcoma is generally a slow growing cancer, there are currently no effective drug treatments. In cases where complete surgical removal is difficult, like the retroperitoneum, recurrence rates and eventual lethality may approach 90%.[10] The development of agents that target CDK4 and MDM2 is proceeding rapidly, with clinical trials of both underway in the US and elsewhere. It is highly likely that the first access to these agents over the next five years will be through clinical trials, which will become a de facto standard of care.

The eligibility criteria for clinical trials are also changing, with an increasing emphasis on the molecular genetics of cancer. Not only does this define potential suitable cancers that have a high a priori chance of benefit (eg. using imatinib in cancers with KIT mutations), but also may help to screen cancers unlikely to benefit. Sarcomas illustrate this point. The p53 pathway is probably the single most commonly mutated in all cancers. Parenthetically, the discovery of TP53 as a tumour suppressor gene was in part made through the study of inherited cancer syndromes (the Li-Fraumeni syndrome). Sarcomas comprise the single most common cancer observed in these unfortunate families, 70% of whom carry in their germline mutations in the TP53 gene.

The p53 pathway has three main components: p14ARF, MDM2 and p53 itself. Different components are inactivated in different cancers. As noted above, MDM2 is amplified in almost all well-dedifferentiated liposarcomas. It appears that MDM2 inhibitors may not work in cancers with mutant p53, because they depend on this gene being functional in order to work.[11] Similarly, in colorectal cancer, KRAS mutations predict for poor response to EGFR inhibitors.[12] Thus an understanding of the molecular genetics of sarcomas will increasingly be critical to understanding who should go on what trial and why some people unexpectedly don't benefit.

Sarcoma and the genetic tsunami

The past decade has seen astonishing developments in our understanding of the molecular genetics of cancer. In 2001, the publication of the human genome sequence heralded a new era in the depth of our mapping of human genetics.[13] This herculean effort, led by Francis Collins of the National Institutes of Health, involved hundreds of scientists across several continents, is estimated to have cost $2.7 billion, and took over a decade to come to fruitition. In the past 10 years, technologic advances in sequencing have resulted in the ability to sequence an entire human genome for under $50,000 and within one month. The genetic mapping of cancer is now being accelerated through 'Big Science' consortia, exemplified by the International Cancer Genome Consortium, whose objective is to fully sequence 500 tumours of each cancer type, beginning with common cancers. Inevitably, the generation of data from such studies will strip away the simplicity of long-held concepts of human genetics. Already, so-called 'junk' DNA is known to be actively transcribed, and to play important roles in development, physiology and disease. The loss of innocence can only continue.

It is highly likely that we will discover new opportunities for therapeutic intervention. These opportunities will include the development of novel agents and strategies for drug development, but they will also include the unexpected discovery of opportunities for application of existing drugs. In this way, the wave of molecular genetics that will emerge in the next decade or more will radically change the textbook treatment of many subtypes of sarcomas. The challenge will be to translate these opportunities into clinical benefit for our patients as quickly as possible. I believe that the rate limiting component of translation of genetics and therapeutics of cancer into clinical benefit will be reform of the health care system, including ethics, processes for clinical trials development, approval and funding of new drugs, and access in the public and private health care systems across our community.

References

1. Australian Institute of Health and Welfare Cancer Data Cubes [Internet]. Available from: http://www.aihw.gov.au/cancer/data/index.cfm [cited 2010 September 20].
2. Braiteh F, Kurzrock R. Uncommon tumors and exceptional therapies: paradox or paradigm? Mol Cancer Ther. 2007;6:1175-1179.
3. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al.The consensus coding sequences of human breast and colorectal cancers. Science.2006; 314:268-274.
4. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004;350:2129-2139.
5. Fletcher DM, Unni KK, Mertens F; World Health Organization. Pathology and Genetics of Tumours of Soft Tissue and Bone. Lyon: IARC Press;2002.
6. Fletcher CD, Gustafson P, Rydholm A, Willen H, Akerman M. Clinicopathologic re-evaluation of 100 malignant fibrous histiocytomas: prognostic relevance of subclassification. J Clin Oncol. 2001;19:3045-3050.
7. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science; 1998;279:577-580.
8. Cassier PA, Ducimetière F, Lurkin A, Ranchère-Vince D, Scoazec JY, Bringuier PP, Decouvelaere AV, Méeus P, Cellier D, Blay JY, Ray-Coquard I. A prospective epidemiological study of new incident GISTs during two consecutive years in Rhône Alpes region: incidence and molecular distribution of GIST in a European region.A prospective epidemiological study of new incident GISTs during two consecutive years in Rhône Alpes region: incidence and molecular distribution of GIST in a European region. Br J Cancer. 2010;. 103:165-70.
9. Moore M, Desai J.New Drugs in the Management of Sarcoma. Cancer Forum. 2010; 157-163.
10. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med. 2002;347:472-480.
11. Thomas DM, Wagner AJ. Specific targets in sarcoma and developmental therapeutics. J Natl Compr Canc Netw. 2010;(8,quiz 686):677-685.
12. Tol J, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG, et al.Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med. 2009;360:563-572.
13. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature. 2001;409:860-921.