IS GENOMICS MAKING RARE CANCERS COMMON AND COMMON CANCERS RARE?

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Abstract

While rare cancers account for a large percentage of total cancer cases, there has been little improvement in the overall survival rates for patients with rare cancers. This can partially be attributed to the difficulties in conducting clinical trials for rare cancers, since it can be challenging to accrue sufficient patient numbers of a rare subtype to demonstrate drug efficacy. A number of rare cancer types however, share genetic drivers with common cancers, which can be therapeutically targeted. Therefore, if patients are stratified by the molecular and genetic characteristics of their tumour, instead of stratifying them by their tumour type, targeted medicine could be a more achievable aim. In order to classify cancers by their genetic composition, we need to perform complex genomic testing, utilising next-generation sequencing technology. In this review, we discuss the screening approaches available for implementing such tests and the challenges that come with them, with an example of a small gene panel used in the Australian Ovarian Cancer Assortment study.

While cancer types prevalent below six cases per 100,000 people per year are classified as rare, the combined frequency of all rare cancers accounts a significant proportion of total cancer cases, around 22%.[1] Due to their individual rarity, rare cancers have been less well studied than common cancers. As a result, there are fewer proven effective therapies and, consequently, poorer overall survival rates. Recent studies have shown that many rare cancers are more likely to have less complex genomes, with several possessing highly specific dominant driver mutations that offer new therapeutic targets and treatment opportunities. This common characteristic helps unite the individually rare cancers into a collective of different tumour types that may benefit from a shared molecularly - directed approach to diagnosis and treatment.

One of the greater obstacles we face in improving outcomes for patients with rare cancers is the traditional model for conducting clinical trials, where typically, large numbers of patients are required in order to prove drug safety and efficacy, and to demonstrate improvement over standard treatments in a given patient population. Due to their rarity, it is difficult, and not infrequently impossible, to accrue sufficient numbers of patients with rare cancers in order to demonstrate a statistically significant improvement.[2] Consequently, most rare cancer types lack proven treatments that have been developed specifically for the particular tumour. Instead, many rare cancers are treated in the same manner as their more common counterparts, which completely ignores their unique genetic makeup, biology and response to treatments. This problem becomes even more apparent when the cancer type is further stratified by the molecular mechanisms into even smaller subgroups. Indeed, the majority of cancer types are highly heterogeneous, meaning that most common cancers are in fact a collection of rare molecular subtypes.[3] So, with the emergence of routine molecular screening and molecular subclassification, most common cancers are also going to become rare. Learning how to deal with rare cancers can, therefore, teach us how to better manage all cancer types.

An example of a rare tumour type, helping to identify new treatment options for a more common tumour type, is high-grade serous ovarian cancer. This is the most common lethal subtype of ovarian cancer, which for decades set the standard of care for most other types of ovarian cancer, despite their obvious differences.[4] The term ‘serous’ denotes that the cell type resembles the cells that normally line the fallopian tube and their finger-like projections, the fimbria, that help capture the ova as they are released from the ovary.[5] This distinguishes this type from other types that arise from the endometrium, germ cells and ovarian stroma. The term ‘high grade’ refers to the aggressive behaviour and degree of nuclear atypia exhibited by this subtype which is a manifestation of underlying genetic changes, mostly TP53 mutations, that characterise this subtype, Acquired TP53 mutations largely distinguish high grade serous tumours from the more indolent ‘low grade’ subtype that has a different set of molecular changes, notably in the MAP Kinase pathway. Approximately 50% of all high-grade serous ovarian cancers have defects in a DNA repair mechanism known as homologous recombination (HR).[6] Until recently, it was thought that most HR-deficient tumours were caused by germline mutations in the breast cancer susceptibility genes, BRCA1 and BRCA2. However, extensive studies of the genomes of different tumour types have shown

that germline, somatic and epigenetic changes in many of the genes that encode proteins that form the HR DNA repair complex can also lead to HR deficiency.[7] Indeed, mutations in these other HR genes increase the proportion of HR-deficient ovarian cancer from 18%, caused by inherited BRCA1 or BRCA2 mutations, to 50%. Furthermore, these additional HR genes are also inactivated in some breast, peritoneal, pancreatic, prostate and probably several other cancers.[8-11] The unrelated observation that HR-deficient tumours, which are unable to repair double strand DNA breaks, are more sensitive to platinum based chemotherapy (which causes double strand DNA breaks) and are uniquely sensitive to PARP inhibitors, which prevent HR-deficient but not HR-proficient cells from repairing the DNA damage,[12] has opened up promising new therapeutic options for not only patients with high-grade serous ovarian cancer, but also potentially other more common HR-defective tumours.

The next challenge to improving outcomes for patients with rare cancers is developing new diagnostic tools to screen tumours for clinically relevant genetic abnormalities. Sanger sequencing has been the method of choice for mutation detection by diagnostic laboratories, where it is ideally suited to screening single genes for inherited or acquired mutations. However, Sanger sequencing is not scalable and becomes a very expensive and time-consuming process when screening multiple genes. In the last decade, the development of next-generation sequencing (NGS) has improved sequencing efficiency many thousand-fold and now provides a low-cost and high throughput approach for performing large-scale genomic analysis in a clinical setting.

While NGS has opened up a lot of opportunities to perform more complex genomic testing, there are still a number of difficulties in utilising it as a comprehensive genomics analysis tool in a diagnostic setting. Firstly, it is still expensive to perform deep whole-genome sequencing in order to ensure that all regions of the genome are properly covered, especially in a cancer genome, where polyploidy, intra-tumour heterogeneity and purity of the sample can cause additional difficulties.[13] Secondly, the amount of data generated by sequencing whole genomes is overwhelming and requires expensive storage.[14] Thirdly, the analysis of large-scale sequencing data is complex and requires highly-skilled bioinformaticians to make sense of the data and experienced medical geneticists to interpret its clinical significance.[15] Finally, even when the data is of high quality and is analysed appropriately, the interpretation of the results in a clinical context can be very difficult, as we are still learning about the function of large regions of the human genome. However, NGS technology is also able to interrogate specific genomic regions of interest with great depth and accuracy. This approach is being rapidly adopted in a diagnostic setting and has the potential to transform the way in which rare cancers are diagnosed, classified and treated.

Small gene panels

Moving forward from single gene tests performed by Sanger sequencing to whole-genome sequencing can be done in stages. By developing small gene panels (5-100 genes), which are affordable in a clinical setting and relatively easy to analyse, we can start covering cancer types that share common mutations, genes or pathways.[16] The early panels tended to capture oncogenes with dominant activating mutations that either conferred drug sensitivity such as EGFR mutation and EGFR tyrosine kinase inhibitors in lung cancer or, resistance exemplified by KRAS mutations and EGFR monoclonal antibodies in colorectal cancer. More recently, panels designed to capture multiple genes that can inactivate common drugable pathways are emerging. As mentioned earlier, the HR pathway is an ideal candidate because a panel can be used to screen tumour samples for mutations that confer sensitivity to PARP inhibitors. The ability to quickly, accurately and rapidly screen tumour samples from a large number of patients with a rare tumour type will greatly increase the pool of patients potentially eligible for enrolment in a clinical trial.

The Australian Ovarian Cancer Assortment Trial is an example of how small gene panels may benefit rare cancers. The project is designed to develop a NGS diagnostic tool that would help to stratify patients with ovarian cancer into treatment categories based on the molecular composition of their tumours. This project not only aims to look at the most common subtype of ovarian cancer (high-grade serous), which accounts for 70% of all ovarian cancer cases, but also to capture molecular events that occur in the rarer subtypes of ovarian cancer, including low-grade serous, mucinous, endometrioid, clear cell, granulosa and dysgerminoma subtypes. A panel of 29 genes, which are known to be mutated in these subtypes of ovarian cancer and can be potentially therapeutically targeted, was developed for screening by NGS technology.

The initial aim of the project is to determine the feasibility and acceptability of this new molecular screening approach before introducing the test into routine care. It is important to introduce this new approach under ethical research guidelines to ensure that the assay is properly validated and accredited, and that only appropriate patients are tested in order to minimise any harm to patients caused by unforseen risks, such as the generation of false results or false hope, and inadvertent delay in obtaining standard of care therapy. The initial phase aims to screen 60 patients with advanced ovarian cancer irrespective of the subtype, with a goal of stratifying them into various treatment groups. It will provide insight into the utility of small gene panels as a diagnostic tool for ovarian cancers. So far, 13 cases have been screened, with most containing at least one clinically significant mutation. Several cases have shown an unexpected degree of complexity, resulting in difficulties and delay in test interpretation. However, we are hopeful that with more exposure to tests like this, it

will become easier to understand tumour progression and resistance mechanisms, and to determine the most suitable treatment approaches.

Genome, exome and sub-exome sequencing

At the other extreme from single gene tests is whole genome sequencing. Since this covers all three billion bases of the human genome, it has the potential to reveal all genetic changes within a tumour. However, this is enormously complex and, currently, way beyond the means and scope of routine diagnostic laboratories, and would not be a judicious use of scarce healthcare resources.

Whole-exome sequencing provides sequencing data for all coding regions of the genome, which is approximately 1/1000 of the scale of whole genome sequening. Sub-exome sequencing uses similar technologies, but focuses on specific areas of the exome. Several commercial panels are now available that target the coding regions of only those genes known to be associated with human disease. Such 'clinical exomes' are likely to become the mainstay of diagnostic genetics laboratories for the analysis of rare diseases, as they are likely to provide the most cost-effective way to interrogate the relevant parts of the genome that will allow the consolidation of potentially hundreds or thousands of individual genes or disease-specific tests into a single platform. Being a universal test that can be used to screen any type of common or rare cancer, it should provide simplicity to diagnostic laboratories, where a single test can be used to detect the majority of molecular abnormalities irrespective of the prevalence of the tumour in the community.

The small gene panels that cover common actionable mutations in common cancer types are likely to become the most cost-effective front line diagnostic test for patients with cancer. The clinical exome is likely to become the second-line test for rare cancers (in which the rare disease-specific mutation may not be captured in a small panel) and in patients whose tumours contain complex pathway alterations, such as patients whose tumours have progressed following multiple rounds of chemotherapy. A number of studies have already employed this sub-exome sequencing approach for classification of rare cancer types.[17,18]

There are however, still a number of hurdles that need to be overcome in order for sub-exome and whole-exome sequencing to become a routine diagnostic test for screening cancers. These technologies do not capture many other genetic alterations (e.g. rearrangements, promoter mutations) or changes in gene expression, and methylation. They also reveal many genetic alterations that are of unknown clinical significance. It is not uncommon to identify thousands of such alterations in a single tumour, many of which have not been previously described. It is therefore going to be a huge challenge to pinpoint an unexpected but key targetable alteration in each case. Improving our ability to accurately predict the significance of novel or rare events is going to require the establishment of global databases in which this information can be shared and interrogated. It is likely that many of these alterations will be shared across multiple tumour types as they invariably affect universal pathways that regulate cell growth rather than lineage determination. Accordingly, rare tumours and rare molecular subtypes of common tumours are going to be increasingly classified according to their therapeutically relevant pathways rather than their organ or presumed cell of origin.

Paradoxically, genomic technologies are making common cancers rare (by subclassifiying them into smaller subtypes) and rare cancers common (by grouping them together into common treatment categories). Hopefully, by improving diagnosis and identifying targeted treatment options we can make both common and rare cancers rare in our communities.

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