Tumour heterogeneity in pancreatic cancer

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Summary

Cancer is a relentless disease defined by uncontrolled cell division. In pancreatic cancer, cells of the pancreas grow and divide rapidly. Cancerous cells can invade and destroy adjacent healthy tissue. Tumours are comprised of cancerous and non-cancerous cells and the original tumour is referred to as the primary tumour. As the disease progresses, different cells in the tumour acquire different mutations resulting in the main body of the tumour consisting of cells possessing molecular differences termed tumour heterogeneity. Pancreatic cancer is an aggressive disease with a high mortality rate. Various pancreatic tumour subtypes have been suggested and as such a 4 type classification system has been devised which includes the following pancreatic tumour subtypes: squamous, pancreatic progenitor, immunogenic and aberrantly differentiated endocrine exocrine. These subtypes are each involved in slightly different genetic processes. Some of the subtypes are associated with a more severe clinical outcome than others such as the squamous subtype which is linked to metastatic disease. Metastatic disease is when the cancer spreads from the primary tumour to other tissue forming a secondary tumour. There are few treatment options available for pancreatic cancer. However, studies have looked at how cancerous cells communicate with each other and identified potential therapeutic sites in these cell communication pathways. Cells from 3 metastatic sites (lung, liver and peritoneum) have been analysed and a cell surface receptor involved in cell communication called Axl was found to be hypophosphorylated in cells derived from lung and liver metastatic cell lines but not in cells from the peritoneal cell lines. Cells derived from these different metastatic sites could possess varying levels of sensitivity to drugs that inhibit these cell communication pathways from working properly. These drugs are called pathway inhibitors and examples include R428 and lapatinib. Tumour heterogeneity in pancreatic cancer provides problems for clinicians and the treatment of the disease. However, a greater understanding of this heterogeneity could lead to the discovery of novel therapeutic targets.

Cancer is defined as uncontrollable cell proliferation (1) as it is a disease involving relentless clonal expansion of somatic cells to result in the killing of 'healthy' tissues through cell invasion and erosion (2). Cancer is thought to be a dynamic disease due to the fact that as the disease progresses the tumours will tend to become more heterogeneous which eventually results in the main body of the tumour consisting of multiple cell-types with differing molecular signatures which have variable levels of sensitivity to treatment (3). Tumour heterogeneity can be divided into intratumoural heterogeneity and intertumoural heterogeneity. Intratumoural heterogeneity defines the heterogeneity within the tumour cells of one patient. These differences between tumour cells may have arisen from phenotypic, genetic and epigenetic features (3).

For the purposes of this review article, I will define intertumoural heterogeneity as the heterogeneity between tumours in different patients where the tumour is of the same stage and grade. Intertumoural heterogeneity arises from patient specific factors such as differing somatic mutation profiles and genetic variation in the germline cells and environmental factors (3). Cancers can spread locally from the primary tumour into the surrounding healthy tissue. This disease is also able to spread to other regions in the body via the blood and lymphatic system to form new tumours. This is known as metastasis and it has been proposed that metastatic tumours may be of the same type as the primary tumour (5). Intermetastatic heterogeneity also exists due to the possibility of metastatic lesions being comprised of different cell populations from the primary tumour. Another possibility is intrametastatic heterogeneity which arises due to the metastatic lesion's ability to obtain new mutations with each round of cell division (3). These differences between inter and intra heterogeneity are all outlined in Figure 1.

As defined previously, intratumoural heterogeneity is the heterogeneity found within tumour cells of an individual patient. Intratumoural heterogeneity is further segregated into spatial or temporal heterogeneity as

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demonstrated in Figure 2. Spatial heterogeneity defines the unequal distribution of genetically different tumour subpopulations located within a lone tumour or across multiple tumour sites. Temporal heterogeneity differs from the former since it describes the variability of the genetic diversity of a single tumour over a time period (6).

Tumour heterogeneity causes numerous clinical implications that can have an impact on the prognosis of the patient. Tumour heterogeneity results in tumours consisting of multiple cells with varying molecular signatures, each with varying levels of sensitivity to treatment. Due to this, resistance to targeted therapies can arise from the evolution of drug tolerant cells (3). Subsequently, there has been a shift in treatment to a more personalised and genotypically guided approach which takes into consideration the genetically diverse tumour subpopulations both within and between cancer patients (3). In 2012 there were an estimated 8.2 million deaths worldwide from cancer (7). This high mortality rate emphasises the importance of developing efficient treatment options for the various cancers.

The use of circulating tumour DNA as a biomarker in pancreatic cancer

Pancreatic cancer is the eleventh most prevalent cancer in the UK and is the sixth most common cause of cancer death in the UK and it accounted for 6% of all cancer deaths in 2016 (8). Pancreatic cancer has one of the worst prognoses out of all the malignancies (9). Since the early 1990's the prevalence rate of pancreatic cancer has increased by approximatley 15% in the UK (8). At present, the only potentially curative treatment is surgery but only around 15-20% of pancreatic cancer patients have resectable disease at presentation in the clinic (9). Unfortunately, in patients who undergo both surgery and adjuvant chemotherapy the 5 year survival rate is incredibly low – just 16.3%-28.9% (9). This shows that this resective surgery is rarely curative.

The most common cause of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC) which makes up 80% of all pancreatic cancer cases (10). PDAC tumours tend to have a high stromal content which creates a strong hypovascular barrier that is considered to hinder the delivery of chemotherapeutics and encourage aggressive neoplastic cell behaviour (10). Earlier detection of PDAC would lead to a more positive patient prognosis as it could increase the probability of successful treatment and improve survival rates (10).

The diagnosis of pancreatic cancer can involve performing a tissue biopsy but it is possible to achieve false negative results due to the high stromal content which is characteristic of many pancreatic cancers. Alongside tissue biopsies being expensive and potentially being painful for the patient, another potential issue with tissue biopsies is that they only give site specific information and will not provide an accurate representation of the entire genomic landscape of the tumour (10). Pancreatic cancer is highly heterogenous and so it is important to consider these limitations of tissue biopsies when conducting mutational profiling as the results from targeted next generation sequencing would not be reliable due to the heterogeneity (10). A way to possibly combat this is to take multiple tissue biopsies from the PDAC tumour as these will take into account the heterogeneity within the tumour to give an accurate representation of the entire tumour's genomic landscape. An alternative method that is currently being investigated is the use of circulating tumour DNA (ctDNA) taken as a liquid biopsy. CtDNA is derived from tumour cells undergoing necrosis or apoptosis which is occurring in the natural development of the cancer from a primary tumour to a metastatic lesion (10). Therefore,

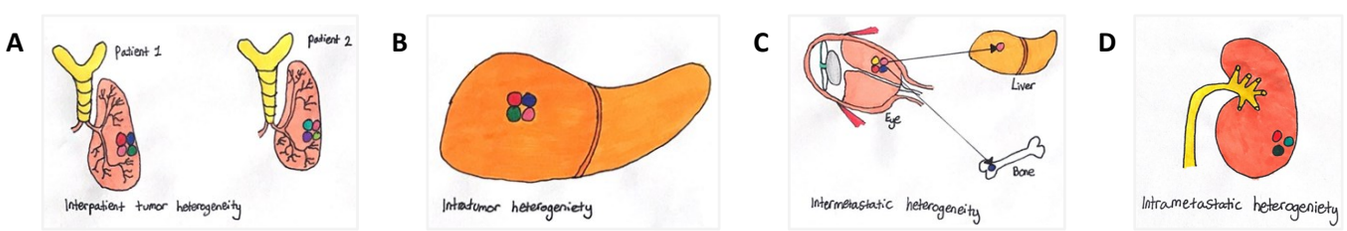

Figure 1. A comparison of intertumoural heterogeneity and intratumoural heterogeneity. A: Intratumoural heterogeneity which is the existence of numerous sub clones in a primary tumour. This results in heterogeneity amongst tumour cells. B: Intratumoral heterogeneity which is heterogeneity among the tumour cells of an individual patient. C: Intermetastatic heterogeneity which is the existence of differing sub clones in separate metastatic lesions of one patient. D: Intrametastatic heterogeneity which is the occurrence of numerous sub clones within a single metastatic lesion. The distinct coloured dots signify the different sub clones. Figure adapted from Jamal-Hanjani *et al.* (4).

ctDNA is thought to be less affected by intratumour heterogeneity than a single tissue biopsy is. This ctDNA also allows for rapid evaluation of tumour changes in a matter of hours since the half-life of ctDNA is approximately 2 hours. This makes the use of liquid ctDNA biopsies ideal for monitoring the effectiveness of treatment and disease progression (11). Furthermore, liquid biopsies are more cost effective, quicker to perform, more comfortable for the patients since only a blood sample is taken and they are easy to repeat.

The use of a liquid biopsy containing ctDNA allows for detection of circulating tumour derived biomarkers in the blood which is a non-invasive approach that could provide an earlier detection of PDAC and other pancreatic cancers (10). These circulating tumour derived biomarkers such as point mutations in key cancer genes can be detected by targeted amplicon sequencing of circulating free DNA (cfDNA) that has been isolated from blood plasma (10). Once these findings have been validated, these circulating tumour biomarkers can be used to detect early PDAC and potentially even premalignant lesions (10). This is hugely beneficial as patient prognosis can be improved through the use of a more effective treatment that is targeted to the genomic landscape of the patients' tumours.

As shown above, tumours can be quantified through the use of liquid biopsies of patient blood. Liquid biopsies contain ctDNA to allow for the detection of circulating tumour derived biomarkers such as mutations of key cancer genes (KRAS, CDKN2A, SMAD4 and TP53). Therefore, in order to quantify tumours by their molecular subtype through liquid biopsies, it will be necessary to detect these circulating tumour biomarkers for each of the 4 molecular subtypes. Each of the 4 molecular subtypes have differing molecular signatures and so it is important to detect the mutations responsible for these differences and utilise them as biomarkers.

Molecular subtypes of pancreatic cancer

Analysis of the genomic landscape of pancreatic cancer has revealed a varied mutational landscape with four common oncogenic mutations in the cancer genes: KRAS, CDKN2A, SMAD4 and TP53 alongside other less commonly mutated genes (12). It has been discovered that oncogenic point mutations of individual genes accumulate into essential molecular pathways which include cell cycle regulation, axonal guidance, DNA damage repair, chromatin regulation and TGF-β signalling.

In a paper by Bailey *et al.* (12), 382 pancreatic cancer patients and their associated histopathological variants were analysed through deep exome sequencing, whole genome sequencing and copy number analysis in order to identify genomic events and key mutations that contribute to disease progression. The study participants had suffered from PDAC and its associated histological variants which were colloid, PDAC associated with intraductal papillary mucinous neoplasm (IPMN), adenosquamous and acinar cell carcinomas (12). Adenosquamous carcinoma, acinar cell carcinomas and colloid carcinoma are rare variants of pancreatic cancer (13). Adenosquamous carcinoma is considered to be more aggressive than PDAC and as a result, patients with this histological subtype have a poorer prognosis (14). It has been found that acinar cell carcinoma tumours show varying morphological characteristics, molecular signatures, patient prognosis and clinical symptoms at presentation in the clinic (15). This varied heterogeneity in the clinicopathological spectrum produces difficulties for the pathological diagnosis of the tumour which in turn has implications on the efficiency of treatment and patient prognosis (15).

The patients who participated in the Bailey *et al.* (12) study were treatment naïve and had undergone resective surgery. Analysis

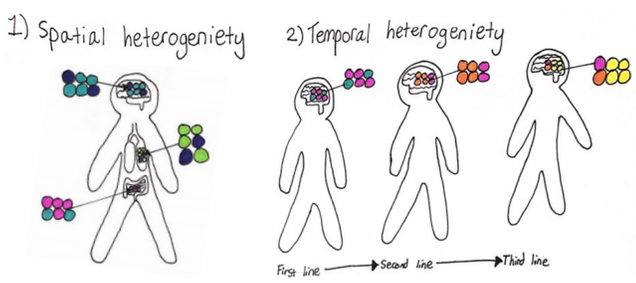

Figure 2. A comparison between spatial and temporal heterogeneity. 1 depicts spatial heterogeneity which is shown by the uneven distribution of cancer sub clones across different regions of the metastatic sites and primary tumour. 2 illustrates temporal heterogeneity which is depicted by the change in the genomic landscape of a single lesion over time due to the tumour's natural progression or exposure to selective pressure such as chemotherapy or immunotherapy. The individual coloured dots signify different sub clones. Figure adapted from Dagogo-Jack *et al.* (3).

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identified 23,538 coding mutations and 7,377 of these mutations were verified through a statistical approach. 21,208 genomic rearrangements were also identified (12). Further analysis of bulk tumour tissue was undertaken in order to gain an understanding of the molecular mechanisms and transcriptional networks that underline the tumour microenvironment. Unsupervised clustering of RNA-seq data for 96 tumours identified 4 molecular classes which were: Squamous, pancreatic progenitor, immunogenic and aberrantly differentiated endocrine exocrine (ADEX) (12). These 4 classes were based on the differential expression of transcription factors and downstream targets that are essential for lineage specification and differentiation in pancreas development and regeneration (12). An alternative proposal for the molecular subtypes of pancreatic cancer is outlined by The Cancer Genome Atlas Research Network and it confirmed 2 pancreatic cancer tumour subtypes which were: classical/ pancreatic progenitor and basal-like squamous (16).

The squamous subtype is characterised by four core gene programmes which includes gene networks involved in metabolic reprogramming, hypoxia response, inflammation, autophagy, MYC pathway activation, TGF-β signalling and upregulated expression of TP63ΔN and its target genes (12). The pancreatic squamous subtype is associated with mutations in KDM6A and TP53. TP63ΔN in the presence of mutated TP53 regulates epithelial cell plasticity, tumorigenicity and epithelial to mesenchymal transition in numerous solid tumours (12). This particular subtype has a notably poor prognosis (17). The pancreatic progenitor subtype is defined by transcriptional networks containing the following transcription factors: HES1, PDX1, FOXA3, FOXA2, MNX1, HNF1A, HNF1B, HNF4G and HNF4A (12). These transcription factors play a hugely important role in pancreatic endoderm cell-fate determination towards a pancreatic lineage (12). The ADEX molecular subtype is characterised by transcriptional networks that play a key role in the latter stages of pancreatic development and differentiation (12). The ADEX subtype is also a subtype of pancreatic progenitor tumours (12). The final subtype is the immunogenic subtype which shares numerous characteristics with the pancreatic progenitor class but it differs in the fact that it exhibits a notable immune infiltrate (12). The immunological genes associated with the immunogenic subtype are genes involved in antigen presentation, toll-like receptor signalling pathways, B cell signalling pathways, CD8 + T cell and CD4+ T cell (12).

Molecular heterogeneity in pancreatic cancer

Recent large-scale genomic studies have identified the transcriptomic and genomic landscape of pancreatic cancer. However, not much is known about the molecular heterogeneity that results from differing tumour locations in the pancreas (17).

The pancreas is located deep in the upper abdomen. It consists of 4 main anatomical structures as shown by

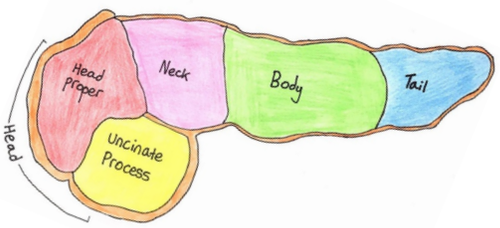

Figure 3. The anatomical structures of the pancreas. The pancreas can be divided into 4 main parts; the tail, the body, the neck and the head which can be further divided into the head proper and the uncinate process. Figure adapted from teachmeanatomy (18).

Figure 3. These structures are: The tail, body, neck and head which can be further segregated into the uncinate process and head proper (19). Studies have revealed notable differences in the prognosis of cancers situated in the head of the pancreas compared to those located in the tail and body (17). Around 15% of PDAC arise in the tail and body of the pancreatic tumour which are characterised by a late presentation in the clinic. Patients with PDAC in the body and tail display symptoms characteristic of advanced disease such as pain and extreme weight loss. Tumours of the pancreatic head are often presented far earlier in the course of the disease since some patients with these tumours often have a comorbid diagnosis of jaundice (17).

Studies have identified that PDAC harbours substantial interpatient genomic heterogeneity. Whole genome sequencing performed on 100 resected PDAC tumours showed novel structural variation subtypes defined by chromosomal rearrangement patterns and numbers which are able to predict the reaction to platinum based chemotherapy through synthetic lethality (15). The resulting mutation produced from the perturbation of two genes by synthetic lethality produces a vulnerability that can be targeted therapeutically (19).

The relationship between body and tail pancreatic cancers and the squamous molecular subtype

Through mRNA microarray sequencing and whole-transcriptome sequencing it has been identified that pancreatic cancers of the tail and body co-segregate with the squamous molecular subtype (17). It has been discovered that there are significantly poorer clinical outcomes for the advanced and resectable disease stages in both tail and body pancreatic cancer (15). A study by Dreyer *et al.* (17) has identified molecular differences amongst resectable PDAC from head, body and tail of the pancreas. The squamous molecular subtype seems to be more advanced on the molecular clock compared to the other molecular subtypes which suggests a further level of genomic instability which results in the accumulation of

DNA damage that influences the novel transcriptome of tumours with this subtype. Therefore, it seems that body and tail pancreatic cancer has a greater chance to be of a squamous subtype thus insinuating a more aggressive disease at the time of diagnosis than cancer of the pancreatic head (15). Dreyer *et al.* (17) defined the squamous molecular subtype on the basis of metastasis of the liver, this metastasis of the liver was associated with a considerably poorer prognosis when compared to additional local and metastatic recurrence patterns.

Metastatic disease in pancreatic cancer

Pancreatic cancer is an aggressive malignancy with a five year mortality rate of 97%-98% (20). This high mortality rate is usually as a result of widespread metastatic disease (20). Metastatic disease is the most frequent cause of death in cancer patients (22). Tumour heterogeneity can be studied in patients with multiple sites of metastatic disease (21). Kim *et al.* (21) hypothesised that heterogeneity in metastatic pancreatic cancer causes heterogeneity at the proteomic level. At the proteomic level it was found that a class of cell surface receptors had a highly altered expression amongst the three metastatic cell lines examined (liver, peritoneum and lung). In this study there was a particular focus on the changes in tyrosine phosphorylation based signalling pathways amongst clones (21). Mass spectrometry was undertaken on purified tyrosine phosphorylated peptides that were enhanced using anti-phosphotyrosine antibodies. Differential activation of the tyrosine kinases was observed in the 3 metastatic subclones (21). Axl receptor tyrosine kinase was hypophosphorylated in the liver and lung metastatic cell lines but not in the peritoneal cell line (21). Due to the differing patterns of cell signalling pathways in the subclones it could be possible that tumours derived from separate sites have the potential to have varying sensitivities to pathway inhibitors (21). Tumour cells derived from the lung metastatic cell line showed a greater sensitivity to the Axl inhibitor R428 whereas cells obtained from the peritoneal metastasis showed an increased sensitivity to lapatinib (21).

Discussion

Pancreatic cancer is a highly heterogenous disease that is continually evolving due to the accumulation of new mutations throughout the course of the disease. Pancreatic cancer is an aggressive disease with a high mortality rate. It often presents late in the clinic when the disease is in its advanced stages. There is little in the way of curative treatment aside from resective surgery which is rarely successful in terms of survival as shown by the low 5 year survival rate. One of the main issues with pancreatic cancer is the fact it is often diagnosed in the latter stages of the disease when patients have multiple sites of metastatic disease and treatment options are limited. If the disease could be detected earlier then perhaps it would lead to a more positive prognosis for the patient; research efforts should be focused on screening methods to allow for the earlier diagnosis of patients. If the use of liquid biopsies containing ctDNA became more widespread then changes in a tumour would be able to be rapidly evaluated regularly thus allowing for a more efficient monitoring of the effectiveness of treatment and disease progression. Tissue biopsies can be further improved by taking biopsies from multiple sites in the primary tumour to give an accurate representation of the genomic landscape of the tumour that takes into account any heterogeneity that may exist. It is hugely important to take tumour heterogeneity into consideration when choosing suitable therapeutics. Conventional blanket chemotherapy provides a very crude treatment approach and can lead to resistance to therapeutics arising from drug tolerant cells which can make further treatment difficult. As death from metastatic disease is common in cancer patients, particularly in pancreatic cancer due to no efficient treatment option existing, it seems sensible that treatment options to target the metastatic disease are developed. Studies at the proteomic level have identified differing patterns in the cell signalling between different metastatic cell lines. Tumours derived from varying sites in the subclones have the potential to be sensitive to different pathway inhibitors and this provides another potential therapeutic target. Tumoral heterogeneity provides numerous issues to clinicians, however, it seems as if targeting this heterogeneity may be the key to developing successful treatment options with the use of a personalised genotypically guided treatment based on the genomic landscape of the individual's tumour. However, it is important to remember that the majority of cancers will become resistant to these targeted therapies. Creating a dual treatment approach that comprises of a targeted approach to the drug tolerant cells and a personalised approach that takes into account the tumoral heterogeneity could lead to huge advances in the treatment of pancreatic cancer. Overall, having more of an understanding of heterogeneity in pancreatic cancer could lead to the discovery of more therapeutic targets.

You might also be interested in the research article by Rebecca Cleator on the interactions between chemotherapy agents used to treat pancreatic cancer: see Research, page 30