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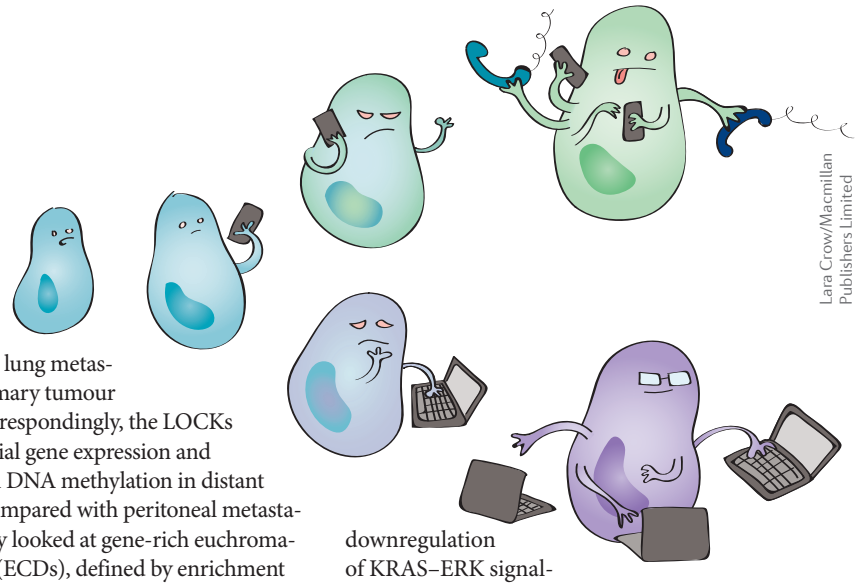
TUMOUR EVOLUTION

Epigenetic and genetic heterogeneity in metastasis



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How similar are metastases to the primary tumour and other metastases in the same patient? And what does that tell us about the evolution of metastatic ability? Two papers investigated these questions in pancreatic ductal adenocarcinoma (PDAC).

Makohon-Moore, Zhang, Reiter *et al.* carried out whole-genome sequencing (WGS) of 39 samples from four patients with stage IV (metastatic) PDAC to generate a picture of the genetic driver alterations in primary tumours and metastases from various organs. They identified 614 PDAC-associated mutations, which they used to estimate the heterogeneity between primary tumours and metastases. They found there was less heterogeneity than expected and that primary tumours and metastases were highly similar. Targeted sequencing of 59 spatially distinct samples of the four primary tumours revealed the phylogeny of the tumour and metastatic subclones. They found that no single subclone gave rise to all or most metastases in a patient and all driver genes in metastases were found in all sequenced regions of the primary tumours. This suggests that driver mutations in these PDACs are not highly heterogeneous and metastasis-specific driver mutations could not be identified.

McDonald, Li *et al.* investigated the impact of epigenetic heterogeneity on the progression of PDAC using a panel of matched primary and metastatic PDAC samples from five patients. Immunostaining revealed differences in global epigenetic reprogramming in primary tumour subclones that seed regional (peritoneal) and distant (liver and lung) metastases. Investigating further, they produced 160 data sets of histone modifications, gene expression changes and whole-genome DNA methylation changes from a subset of the primary tumour, regional metastasis and distant metastasis samples. In peritoneal metastasis, histone 3 lysine 9 dimethylation (H3K9me2) was enriched in large organized chromatin H3K9-modified (LOCK) heterochromatin, but these same regions had reduced H3K9me2 in lung metastasis and primary tumour samples. Correspondingly, the LOCKs had differential gene expression and reductions in DNA methylation in distant metastasis compared with peritoneal metastasis. Next, they looked at gene-rich euchromatin domains (ECDs), defined by enrichment for acetylated H3K27 (H3K27ac) and H3K36 trimethylation (H3K36me3) and depletion of modifications associated with heterochromatin. Mapping gene expression data and ECDs revealed local reprogramming such that upregulated genes in ECDs had increased levels of H3K36me3 and H3K27ac. Thus, they found that substantial epigenetic reprogramming occurs at sites of heterochromatin and euchromatin and that this differs between regional and distant metastasis.

> “although there is little heterogeneity in driver mutations…there is considerable epigenetic reprogramming”

Does this epigenetic reprogramming affect tumour cell phenotype? They analysed the ontology of differentially expressed genes in ECDs and LOCKs between matched metastatic subclones from the same patient. The differentially expressed genes in LOCKs from lung metastasis were associated with oxidoreductase activity, and the corresponding cell cultures were resistant to oxidative stress. Moreover, genes encoding epithelial and mesenchymal identity were reciprocally expressed in LOCKs such that peritoneal metastasis was epithelial and lung metastasis was poorly differentiated. DNA repair genes were upregulated from ECDs in lung metastasis, and these cells were resistant to chemotherapy. The lung metastasis also had downregulation of KRAS–ERK signalling from ECDs and was resistant to KRAS-G12V knockdown.

Are there metastasis-specific epigenetic alterations? Cells from distant metastases had high glucose uptake and lactate secretion compared with regional metastasis cells. They also found recurrent depletion of 6-phosphogluconic acid (6PG) in cell cultures of distant metastasis and their precursors. 6PG is the substrate for 6-phosphogluconate dehydrogenase (PGD) and knockdown or inhibition of PGD with the prodrug 6AN reversed the reprogrammed epigenetic state, but had no effect on cells from peritoneal metastasis. Treatment with 6AN also reversed several reprogrammed chromatin modifications in the distant metastasis cells (even after 6AN removal from culture medium) as well as reduced tumorigenicity as measured in various tumour-forming assays, whereas normal and peritoneal metastasis cells were unaffected by treatment with 6AN.

Therefore, although there is little heterogeneity in driver mutations between primary and metastatic PDAC tumours, there is considerable epigenetic reprogramming. In particular, McDonald, Li *et al.* found differences in epigenetic reprogramming between regional and distant metastasis, leading to differences in metabolism and identification of a potential therapeutic target in distant metastasis.

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ORIGINAL ARTICLES Makohon-Moore, A. P. *et al.* Limited heterogeneity of known driver gene mutations among the metastases of individual patients with pancreatic cancer. *Nat. Genet.* http://dx.doi.org/10.1038/ng.3764 (2017) | McDonald, O. G. *et al.* Epigenomic reprogramming during pancreatic cancer progression links anabolic glucose metabolism to distant metastasis. *Nat. Genet.* http://dx.doi.org/10.1038/ng.3753 (2017)