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METASTASIS

Staying organized

> TSIPs can initiate peritoneal metastasis

Although metastasis accounts for most cancer-related deaths, we still have a limited understanding of the biological mechanisms of cancer dissemination. A study published in *Nature Cell Biology* has shown that tumour spheres that maintain an inverted epithelial architecture originate from primary colorectal cancers (CRCs) and can collectively invade the peritoneum, initiating metastasis.

Zajac et al. examined peritoneal effusion samples from patients with CRC with peritoneal metastases and discovered the frequent occurrence of cell clusters co-expressing CRC markers. The majority of these tumour cell clusters were spherical, with clear epithelial organization. However, in contrast to normal epithelial tissues, the tumour spheres had inverted polarity — the apical poles were oriented outwards and basolateral poles faced inwards. These tumour spheres with inverted polarity (TSIPs) were most commonly observed in samples from poor prognosis subtypes of CpG island methylator phenotype (CIMP) CRC (mucinous, micropapillary and cribriform), and their presence was associated with peritoneal metastasis.

Single mucinous CRC cells cultured ex vivo were unable to form TSIPs and died in suspension or culture in extracellular matrix (ECM), whereas isolated TSIPs remained viable. In addition, mucinous CRCs had no evidence of activation of an epithelial-to-mesenchymal transition (EMT) programme, and TSIPs were present in primary tumours, where they were observed to form via a budding process. These data support the hypothesis that TSIPs originate from the primary tumour and do not form from single cells that have undergone EMT followed by mesenchymal-to-epithelial transition (MET).

Gene expression analysis indicated that transforming growth factor-β (TGFβ) signalling was downregulated in mucinous CRCs compared with a CRC subtype that did not produce TSIPs. When treated with TGFβ, a mucinous CRC cell line that undergoes budding when grown in a monolayer showed reduced TSIP production, and various methods of inhibiting TGFβ signalling increased TSIP production. Specifically, inhibition of both SMAD2 and the non-canonical TGFβ effector partitioning defective 6 homologue-α (PAR6α) increased TSIP formation, indicating a role for both of these pathways in TSIP formation.

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The authors next examined whether TSIPs could also undergo collective invasion and initiate metastasis. Ex vivo co-culture of matched TSIPs and peritoneal explants isolated from patients showed that intact TSIPs, which maintain their outward apical orientation even in the presence of ECM, can undergo collective invasion. Non-canonical (but not canonical) TGFβ signalling was required to maintain the inverted polarity of the TSIPs. Collective migration of TSIPs did not require proteins typically involved in collective cell migration (such as focal adhesion kinase (FAK), RAC1 and integrins); this is consistent with the internal basolateral localization of these proteins in TSIPs. Instead, TSIP migration was driven by increased actomyosin contractility induced by non-canonical TGFβ signalling. Finally, TSIPs (but not single CRC cells) injected into the peritoneal cavity of immunodeficient mice formed lesions that maintained inverted polarity, as observed in patient samples, suggesting that TSIPs can initiate peritoneal metastasis.

These data challenge our views of the mechanisms of metastasis. It will be interesting to determine if similar processes occur in other tumour types.

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ORIGINAL ARTICLE Zajac, O. et al. Tumour spheres with inverted polarity drive the formation of peritoneal metastases in patients with hypermethylated colorectal carcinomas. *Nat. Cell Biol.* http://dx.doi.org/10.1038/s41556-017-0027-6 (2018)