COLORECTAL CANCER

Does early metastatic seeding occur in colorectal cancer?

Alessandro Magrì and Alberto Bardelli

During the metastatic process, cancer cells spread from the primary tumour to distant organs. Although metastases are the primary cause of cancer mortality, the dynamics of the metastatic process remain largely unknown. A new study suggests that during colorectal cancer progression, metastatic seeding might occur before clinical detectability.

Refers to Hu, Z. et al. Quantitative evidence for early metastatic seeding in colorectal cancer. *Nat. Genet.* **51**, 1113–1122 (2019).

When a tumour is confined to the organ where it has emerged, such as the breast or the intestine, treatment with surgery is highly effective and often curative. However, when neoplastic cells have already spread to a site distant from the one where they originated the disease is often hard to control. Colorectal cancer (CRC) is the third most common cancer and half of all patients with CRC will develop metastases[1]. CRC is driven by evolutionary processes and develops through a series of stages that have been elucidated in great detail. These stages include dysplasia of the mucosae followed by the formation of adenomas (polyps), which can progress further to more aggressive stages and ultimately to the formation of metastases often to the liver, lung and peritoneum, and less frequently to other body sites such as bone and the brain[2]. The timing and molecular determinants of the metastatic process in CRC are still under debate, but it is generally believed that multiple years, or even decades, are required to proceed from the adenoma stage to the formation of liver metastases[3].

In a new study published in *Nature Genetics*, Hu and colleagues[4] hypothesized that CRC cells depart the primary tumour extremely early, when the disease is clinically undetectable with current methodologies. They postulate that some CRCs are 'born to be bad' — that is, the invasive and metastatic potential of a subset of CRCs is prespecified either genetically or epigenetically as has been previously suggested[5]. To test this possibility, the authors performed whole-exome sequencing on 118 samples from 23 patients with metastatic CRC who had developed metastases in the liver, lung or brain. Multi-region sequencing was performed on the primary tumour and paired metastasis to build phylogenetic trees. Using sophisticated computational methods, the researchers generated patient-specific detailed genetic maps of clonal and subclonal evolution occurring in the primary tumours and matched metastases.

In agreement with previous literature, the study shows that genomic divergence between the primary tumour and the metastasis is relatively low[4]. Indeed, more than 70% of high-frequency somatic single-nucleotide variants were shared between the primary tumour and the metastases. Genomic alterations commonly occurring in CRCs, such as mutations in *APC*, *KRAS*, *TP53*, *SMAD4* and *TCF7L2*, were also highly concordant between primary tumour and metastasis pairs. Of relevance, the study found that some mutations in *SYNE1* and *APOB* genes tended to be 'private' (that is, they occurred mainly in the primary tumour or the metastasis). Furthermore, gene amplification in *PIK3CA*, *GNAS*, *SRC*, *FXR1*, *MUC4*, *GPC6* and *MECOM* seemed to be specific to metastases.

The most innovative section of the study is the phylogenetic analysis of CRC evolution, which indicated early divergence of the metastatic lineages[4]. Using an inference algorithm called SCIMET (spatial computational inference of metastatic timing) the authors simulated the timing of when the metastasis originated from the primary tumour or other lesions. They assumed that CRC initiates from a single founder cell and concluded that the metastasis is seeded by a random single cell from the periphery of the tumour when it is very small (<1 cm^3 in volume). The number of mutations private to the metastasis correlated positively with the timing of dissemination, and subclonal events occurred more frequently than clonal events in metastases, suggesting that CRC dissemination is an early event. According to the SCIMET algorithm, the first metastatic seeding occurred very early in >80% of patients when the primary tumour was below the limits of clinical detectability (FIG. 1). To confirm the findings, Hu et al.[4] used an independent cohort, the MSK-Impact and GENIE public databases which include >2,000 clinically annotated CRC cases[6]. This analysis showed that ~90% of patients with metastatic CRC exhibited subclonal selection consistent with the metastatic clone having a selective growth advantage. By contrast, only 33% of patients with early-stage CRC (stages I–III) exhibited subclonal selection. Interestingly, 43% of distant metastases evolved neutrally, reflecting the high fitness of the metastatic initiating clone.

The results of Hu et al.[4] are consistent with findings from a previous study which reported that private mutation patterns in CRCs might be a marker of early cell movement and of invasive and metastatic potential[5]. In that study, early abnormal cell movement was found in 9 of 15 invasive 'born to be bad' CRCs but in none of four benign adenomas[5]. The work by Hu and colleagues[4] takes another step forward in reporting that alterations in a subset of genes (such as *TCF7L2*, *AMER1* and *PTPRT*) seem to be metastasis-specific, suggesting that they are involved in driving the metastatic process. Given that *PTPRT* encodes an enzyme, if these observations are confirmed, *PTPRT* could act as a biomarker and should be prioritized as a therapeutic target.

The work by Hu et al.[4] concludes that in CRC, metastatic seeding occurs very early in the majority of cases, when the neoplastic mass comprises <10^6 cells, or even fewer. According to the authors' estimates, as few as 10^5 cells (~0.001 cm^3 in volume) can initiate the CRC metastatic process.

As highlighted by the authors, such a small mass is undetectable with current clinical methods. However, and in apparent conflict with their findings, sigmoidoscopy and

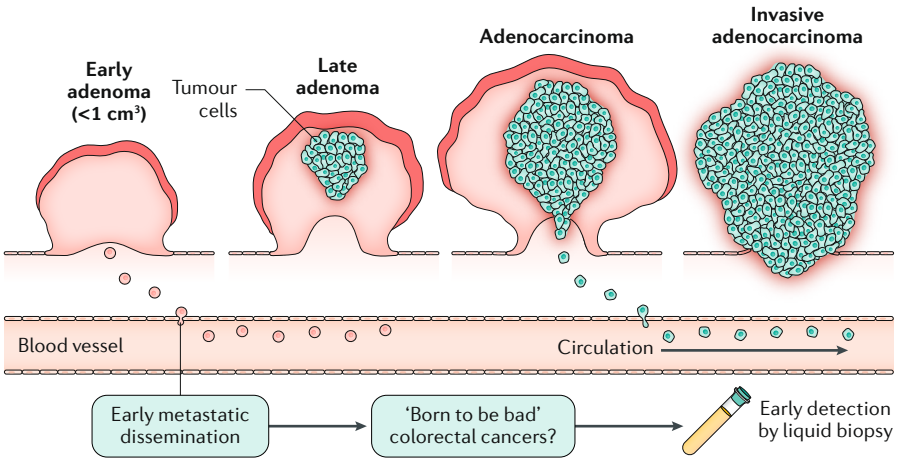

Fig. 1 | **Early metastatic seeding in a subset of CRCs.** A fraction of 'born to be bad' colorectal cancers (CRCs) might carry unique and intrinsic biological traits conferring invasive and metastatic potential before when metastasis was previously considered to occur in the progression of CRC. Identification of these tumours using blood-based assays would be clinically valuable.

colonoscopy are highly successful at detecting early CRC lesions (adenomas) and are currently routinely applied in screening protocols[7,8]. Multiple studies have shown that the detection and removal of adenomas is very effective and reduces CRC mortality. Most individuals in whom an adenoma is detected and removed during routine colonoscopy do not develop localized or metastatic CRC[7,8]. If in the majority of CRCs malignant clones can seed when the initial mass contains so few cells then it is not clear how removal of an adenoma during colonoscopy is so effective considering that adenomas contain >10^6 cells. The authors seem to refer to a subset of cells within an adenoma (the so-called malignant clone), but the efficacy of colonoscopy indicates that even when adenomas reach 10^8 cells (~1 cm^3 in volume, the clinical detection limit) their removal is highly effective and relapse (or metastases) are not often detected 5–10 years after the procedure. As noted by the authors, a considerable number of patients with metastatic CRC do not exhibit early systemic spread. Perhaps, therefore, the fraction of 'born to be bad' CRCs is in fact relatively small.

Up to two-thirds of distant metastases and lymph node metastases originate from independent subclones within the primary colorectal tumour[9]. It would, therefore, be very useful to assess whether 'born to be bad' CRCs metastasize through lymph nodes. Whether the same patterns of early dissemination apply to bowel tumours developing via the serrated pathway, rather than by conventional tumorigenesis, is also unknown. In light of the above points and the limited sample size of Hu et al.'s study, further investigations are required to define more accurately the fraction of CRCs 'born to be bad' as their identification could indeed be clinically relevant.

How could 'born to be bad' CRCs be detected early? One possibility might be combining invasive examinations such as sigmoidoscopy and colonoscopy with blood-based tests such as liquid biopsies, which are aimed at providing accurate molecular profiles of adenomas and other intestinal lesions[10]. If 'born to be bad' biomarkers can indeed be detected in the blood, they would be extremely valuable for identifying individuals at high risk of developing aggressive forms of CRC. This population could receive ad hoc adjuvant strategies such as aggressive chemotherapy to restrict or eliminate the risk of early metastatic dissemination.

Alessandro Magrì[1,2] *and Alberto Bardelli*[1,2]*

[1]*Candiolo Cancer Institute FPO-IRCCS, Candiolo, Italy.*

[2]*Department of Oncology, University of Torino, Candiolo, Italy.*

**e-mail: alberto.bardelli@unito.it*

https://doi.org/10.1038/s41575-019-0200-4

1. Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2019. *CA Cancer J. Clin* **69**, 7–34 (2019).
2. Turajlic, S. & Swanton, C. Metastasis as an evolutionary process. *Science* **352**, 169–175 (2016).
3. Jones, S. et al. Comparative lesion sequencing provides insights into tumor evolution. *Proc. Natl Acad. Sci. USA* **105**, 4283–4288 (2008).
4. Hu, Z. et al. Quantitative evidence for early metastatic seeding in colorectal cancer. *Nat. Genet.* **51**, 1113–1122 (2019).
5. Ryser, M. D. et al. Spatial mutation patterns as markers of early colorectal tumor cell mobility. *Proc. Natl Acad. Sci. USA* **115**, 5774–5779 (2018).
6. Consortium, A. P. G. AACR Project GENIE: Powering Precision Medicine through an International Consortium. *Cancer Discov.* **7**, 818–831 ***CD-17-0151*** (2017).
7. Doubeni, C. A. et al. Effectiveness of screening colonoscopy in reducing the risk of death from right and left colon cancer: a large community-based study. *Gut* **67**, 291–298 (2018).
8. Lauby-Secretan, B. et al. The IARC Perspective on Colorectal Cancer Screening. *N. Engl. J. Med.* **378**, 1734–1740 (2018).
9. Naxerova, K. et al. Origins of lymphatic and distant metastases in human colorectal cancer. *Science* **357**, 55–60 (2017).
10. Siravegna, G. et al. Integrating liquid biopsies into the management of cancer. *Nat. Rev. Clin. Oncol.* **14**, 531–548 (2017).

Acknowledgements
The authors thank Federica Di Nicolantonio for critically reading the manuscript. Research by A.B. is supported by the Fondazione AIRC per la Ricerca sul Cancro under 5 per Mille 2018 (project 21091), AIRC investigator grant 2018 (project 21923) and Fondazione Piemontese per la Ricerca sul Cancro ONLUS 5 per mille 2014.

Competing interests
The authors declare no competing interests.