Beyond traditional subtyping: a multilayered genomic perspective on colorectal cancer

In an article published in April 2022 in *Gut*, the authors introduced an enhancer-based classification system known as EpiC, which facilitated the epigenomic categorisation of colorectal cancer (CRC).[1] Following this, a novel approach was presented in a recent issue of *Nature Genetics*, dated 13 February, by Malla *et al*. This study diverges from traditional gene-centric analysis typically seen in transcriptomics-based classification by incorporating pathway analysis and gene ontology.[2]

The EpiC study posited that tumours from consensus molecular subtype 1 (CMS1) and CMS4 samples, which include those enriched in metabolic and stem cell characteristics, could be categorised into EpiC1 and EpiC2, respectively.[3] The study's enhancer-based approach effectively segregates the CMS2 subtype into two distinct EpiC clusters; EpiC3 and EpiC4 with significantly different survival rates (figure 1). This finding underscores the potential of enhancer-based epigenomic subtyping in offering a better understanding of CRC, which could lead to more efficacious therapeutic interventions. In pursuit of such therapeutic strategies, Malla *et al* embraced an innovative use of the PDSclassifier tool on 165 patients leading to the identification of three distinct subtypes: pathway-derived subtype 1 (PDS1) with upregulated cell-cycle pathways; PDS2, featuring inflammatory and immune signalling pathways and PDS3, characterised by a decrease in stem cell populations and an increase in differentiation, pointing to a poor prognosis in advanced disease. Their analysis showed a significant division of CMS2 samples between PDS1 and PDS3, with the latter associated with the lowest survival rates.

In the CMS molecular subtyping cohort, CMS4 was originally identified as the subtype with the poorest overall survival. However, both the PDS and EpiC classifications have highlighted a subset of CMS2 that exhibits the most adverse survival outcomes. The CMS study associates tumours with stem cell-related features with the worst prognosis. In contrast, studies that explore more detailed genomic signatures—whether by investigating regulatory elements such as enhancers or by employing a pathway-derived approach—reveal a subset of highly differentiated tumour types within CMS2 as having the poorest prognosis. Additionally, the CRC intrinsic subtypes (CRIS) classification, which focuses on the inherent molecular traits of the epithelial components of colorectal tumours, has divided CMS2 into three main subgroups, with CRIS B showing the poorest outcomes.[4 5] Meanwhile, the intrinsic-consensus molecular subtype (iCMS) classification by Joanito *et al* categorises fibrotic-CMS4 as a segment of a broader iCMS2 subtype.[6] This refined insight challenges conventional views and suggests that a multilayered genomic analysis can uncover more accurate prognostic subtypes.

The novel subset of the CMS2 subtype, initially identified as EpiC4 in the enhancer-based subtyping, exhibited neural characteristics, including regulation by SOX9, NPTX2 and NGFR. This pattern was linked to the tumour cells' invasion into the intestinal neural network. In the study by Malla *et al*, a subset of CMS2 tumours displayed signatures of enteroendocrine and enterocyte cells, which are highly differentiated intestinal cells. Although enteroendocrine cells are not directly derived from neural cells, they share neuronal-like properties, such as the ability to produce and secrete neurotransmitters, and they closely interact with the extensive network of neurons in the enteric nervous system. These findings, from two distinct methodological approaches, converge on a similar conclusion: they identify a unique subset within the CMS2 subtype that is associated with the enteric nervous system. Notably, the remaining CMS2 tumours are characterised by MYC enrichment, as observed in both the enhancer-based (EpiC3) and pathway-derived (PDS1) studies.

In summary, over the past decades, various molecular subtyping strategies have laid the groundwork for a foundational classification system that has been continually refined and expanded by incorporating new subtyping methods.[3 7 8] Although these subtyping techniques facilitate targeted therapeutic approaches, they inherently involve some level of arbitrariness depending on the chosen classification method and parameters. Approaches that focus on the functional characteristics of tumours offer a more detailed understanding of each CMS, potentially paving the way for the development of personalised therapeutic strategies.

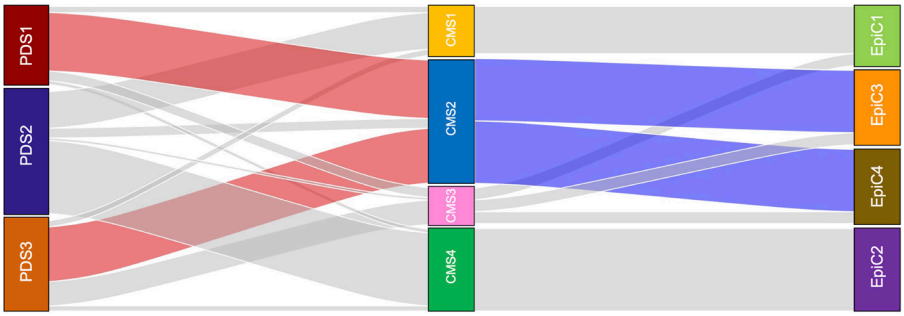

Figure 1 Pathway-derived subtype (PDS) and enhancer-based (EpiC) subtyping of colorectal tumours in association with their original consensus molecular subtypes (CMSs).

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