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Intratumoural Epigenetic Heterogeneity in Early Invasive Colorectal Cancer: A Prognostic Imprint?

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Intratumoural epigenetic heterogeneity in early-invasive colorectal cancer; a prognostic imprint?

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This "Letters to the Editor" submission is in relation to the recent *Gastroenterology* publication:

Martínez-Cardús A, Moran S, Musulen E, Moutinho C, Manzano JL, Martinez-Balibrea E, Tierno M, Élez E, Landolfi S, Lorden P, Arribas C, Müller F, Bock C, Tabernero J, Esteller M, Epigenetic Homogeneity Within Colorectal Tumors Predicts Shorter Relapse-free and Overall Survival Times for Patients With Loco-regional Cancer, *Gastroenterology* (2016), doi: 10.1053/j.gastro.2016.08.001.

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Dear Editor,

We read with interest the manuscript from Martínez-Cardús and colleagues,¹ which defines methylation heterogeneity, both inter-patient (IPH) and intra-tumoral (ITH), using multiregional sampling in colorectal cancer (CRC). The authors show that samples will cluster according to patient-of-origin rather than site-of-sampling. This observation, where only small variations in molecular profiles are found at different tumoral regions, is in agreement with our recent transcriptional-based CRC profiling using multiregional sampling of central tumor and invasive front(IF).²

The authors demonstrate that metastatic tissue displays a higher epigenetic similarity to the inner tract surface (TS) than IF. This is an intriguing finding; one might expect that the IF, containing actively invasive cells giving rise to the metastatic lesion, would share similar traits with metastatic tissue. Their finding suggests that invasion and micro-metastatic disease occur at an early stage. This is particularly relevant given the increasing stage I population being detected due to bowel screening worldwide. As a result of screening, we are for the first time detecting patients with the most aggressive disease, not at stage IV, but while they are loco-regional, presenting a key challenge for identifying patients with the highest risk of metastatic relapse at this stage. Data presented here may inform patient stratification and provide an additional prognostic tool for clinical decision-making in aggressive early-stage disease.

Conversely, the metastatic-TS similarity could also be explained using the phenotypic plasticity model,³ involving transient epithelial-to-mesenchymal transition (EMT). Interpreting the data presented here using this model suggests that the IF will display an increased diversity of epigenetic changes indicative of an ongoing EMT, supported by the high IF diversity noted by the authors. This signaling is reversed during mesenchymal-to-epithelial transition at the metastatic site. Transient EMT signaling shift of the IF could explain the similarity of the metastatic site to the relatively non-invasive TS.

Clinical relevance of epigenetic heterogeneity was tested and tumor stage was identified as an independent prognostic factor; therefore subsequent analyses were limited to microsatellite stable loco-regional stage I/II/III disease (n=50). Using Cox proportional hazard regression, relapse-free survival remained an independent prognostic factor. While removal of stage IV tumors is clearly warranted and pooling of stage II/III is widely used when undertaking analysis of this type, we have concerns relating to the pooling of stage I cases (5-year survival rate >93%), with more advanced stage III tumors, (5 year survival rate 64%, ~50% for stage IIIC). A small numerical imbalance between stage I and stage III cases, and associated relapse-risk, could confound clinical interpretation, even with the appropriate adjusted multivariate model employed by the authors. Additionally, IF type, either expansive or infiltrative, which displays a prognostic trend in the cohort, may confound survival between the identified methylation subtypes. These factors become more pertinent when undertaking stratification in a small pre-filtered cohort of 50 cases.

Although the authors have accounted for tumor/stromal ratio using H&E assessment, we and others have shown that subtle changes in particular cell lineages can influence transcriptomic profiles and have prognostic consequences.^{2,4,5} H&E staining provides a general assessment of different cellular components in each intra-tumoral region, but cannot account for precise changes in specialized tumor/stroma/immune lineages without the use of digital pathology combined with single/multiplexed biomarker staining. Immune population estimates can be made from methylation

data alone,⁶ or in combination with transcriptomics, allowing *in silico* cell-specific enumeration.⁷ Addition of *in situ* or *in silico* profiling for tumor/stromal populations would reinforce the general histology assessment and allow extended interpretation of the epigenetic biology underpinning the findings.

The data presented demonstrate that methylation patterns with the majority of probesets are unaltered across different regional tumor architecture, remaining imprinted according to patient-of-origin. The clinical relevance remains unanswered, as interpretation of methylation-based prognostic signatures are ultimately dependent on defined signaling pathways or in some cases individual genes.⁸ When prognostic classification of methylation is distilled to this level of granularity, the subtle changes observed at different regions may result in misclassification, similar to the issues we reported for transcriptional classifiers based on stromal-derived genes.² This is most relevant in prospective stratification of patients using colonoscopy or biopsy tissue, where the region-of-origin of the sample from within the three-dimensional tumor architecture is unknown. Testing of clinically informative signatures for their robustness following potentially confounding multi-regional sampling is required.

Overall, this report provides additional support for the role of transcriptional and epigenetic changes in early neoplastic development. Generation of a classifier of aggressive early-invasive disease, based on comprehensive biological interrogation, has the potential to immediately inform the management of CRC at the earliest disease stage.

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