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GASTROINTESTINAL CANCER

Turning up trumps for new CRC subtypes

Colorectal cancer (CRC) is a heterogeneous disease, and patients with similar tumour stages have highly variable outcomes. Currently, there are no prognostic or predictive biomarkers to select patients who would benefit from treatment and those who could be spared the toxicity of chemotherapy. Owing to this unmet medical need, two groups of researchers—whose findings have been published in *Nature Medicine*—developed new integrated molecular and drug-response-based classification systems to identify prognostic and predictive biomarkers. They also defined new colon or CRC phenotypes in patients and explored these assays for use in the clinic.

In the first study, led by Louis Vermeulen and Jan Paul Medema, the researchers used an unsupervised classification strategy. Although gene-expression signatures to determine prognosis have already been identified, the genes involved do not seem to overlap, leading the researchers to speculate that these signatures represent different biological subtypes of colon cancer. They derived gene-expression data from 90 patients with stage II colon cancer and used consensus-based clustering to identify patient subgroups using a 146-gene classifier. "The benefit of this approach over previous methods is that we did not use any prior genetic or clinical information; therefore, we have an unbiased separation of patients in distinct disease entities," explains Vermeulen.

The investigators defined three colon cancer subtypes: colon cancer subtype 1 (CCS1) displayed the characteristics of chromosomal instability; CCS2 consisted entirely of microsatellite unstable and CpG-island methylator phenotype-positive tumours; and the completely new CCS3 subtype, which was related to sessile serrated adenomas and was correlated with a very unfavourable prognosis. Importantly, the distinct CCS3 subtype had high expression of genes associated with invasive growth and metastases. "Tumours

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belonging to the CCS3 subtype are primed for invasive growth and metastatic spread, even before they become malignant, which could explain why patients with these tumours often develop a recurrence," says Vermeulen. Notably, the CCS3 tumour subtypes were also insensitive to cetuximab, irrespective of *KRAS* mutational status. Vermeulen concludes: "The fact that the CCS3 tumours are so different from the other tumours immediately suggests exciting avenues to explore in order to optimize selective targeting of this population."

In the second study, Douglas Hanahan and coauthors assessed gene-expression profiles of 1,290 resected CRC tumours, and the resulting classifier was used to associate cetuximab response in 80 patients. "One of the goals was to identify alternative therapies for patients who are not responsive to cetuximab," explains Hanahan, "in addition, we identified unique cellular characteristics in our subtypes that may eventually help to design targeted therapies with lower toxicity for patients in the clinic."

The researchers began with an unsupervised consensus clustering approach using a nonmatrix factorization (NMF) algorithm followed by gene selection using statistical analysis of microarrays. Hanahan explains the rationale for this approach: "we used NMF because it does not impose a hierarchy on gene-expression clusters."

Initially, the team defined five subtypes. They observed that patients from one of the subtypes exhibited a bimodal disease-free survival (DFS) response to cetuximab in the metastatic setting, indicating this subtype was heterogeneous. They split this subtype into two subtypes, which finally yielded a total of six subtypes. "This is a novel approach of subtyping tumours by overlaying drug response over molecular subtypes that has not been tried previously to our knowledge." Three of the subtypes exhibited a better prognosis after resection when left untreated, indicating that these patients might be spared therapy. The poorest DFS was noted in a subtype with stem-like properties, but this subtype also showed the greatest benefit from systemic chemotherapy. Two other subtypes also benefited from FOLFIRI chemotherapy, whereas the subtype that displayed high filamin-A expression (a regulator of MET) is predicted to respond to MET inhibitors.

The researchers used their data to propose possible treatment stratification both in the adjuvant and metastatic settings. Hanahan comments on the significance of the research findings: "our study goes one step beyond previous studies in that we identified unique subtypes with varying responsiveness to clinically deployed therapies, and defined tractable gene signatures to validate subtypes and to predict therapeutic responsiveness. A future application of our current study would be to adopt this classification system in the clinic."

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Original articles: De Sousa E Melo, F. *et al*. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat. Med*. doi:10.1038/nm.3174 | Sadanandam, A. *et al*. A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat. Med*. doi:10.1038/nm.3175