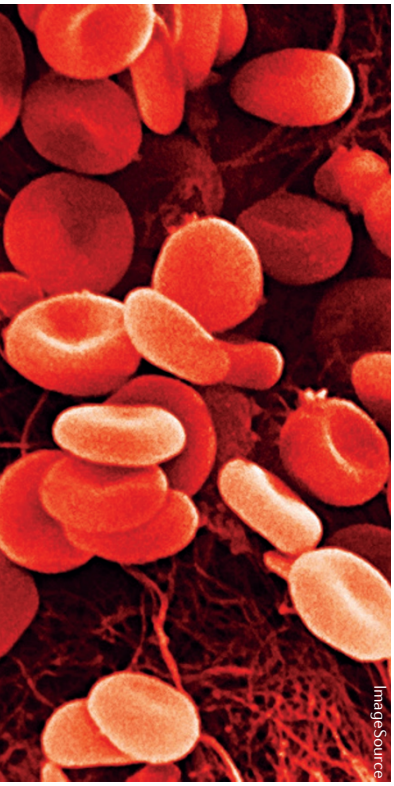
Nature Reviews Cancer | AOP, published online 13 November 2015; doi:10.1038/nrc4048

DIAGNOSIS

RNA-seq for blood-based pan-cancer diagnostics



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A systemic, minimally invasive diagnostic test to determine whether an individual has cancer could greatly improve cancer prognosis by facilitating early diagnosis and improving monitoring. However, the use of blood-based 'liquid biopsies' is challenging, because the biosources (such as plasma DNA, exosomes and circulating tumour cells) and analytical platforms typically have a suboptimal sensitivity. Now, Würdinger and colleagues have developed an alternative method based on their previous observation that blood platelets change their mRNA expression profile in response to contact with a tumour (so-called 'tumour-educated platelets'); combining platelet RNA sequencing with a self-learning algorithm results in a highly sensitive, specific and accurate diagnostic test for cancer.

To establish their method, platelet samples from healthy controls (n=55), patients with early, localized tumours (n=39) and patients with advanced, metastatic tumours (n=189) were collected, covering 6 different tumour types (lung, colorectal, brain, pancreatic, hepatobiliary and breast cancer). SMARTer mRNA amplification followed by sequencing and selection of spliced RNA reads yielded ~5,000 differentially expressed protein-coding and non-coding RNAs between patients with tumours and healthy controls. Selection of a classifier-specific gene list of 1,072 RNAs combined with the use of a custom machine-learning algorithm allowed discrimination of the presence versus the absence of cancer with a sensitivity (proportion of correctly identified positives) of ~97%, a specificity (proportion of correctly identified negatives) of ~94% and an accuracy of ~95%. By contrast, the use of random classifiers had no predictive power. Thus, nearly all cancer patients — regardless of the type of tumour — have abnormal platelet RNA profiles and are identified by the algorithm.

Next, tumour-specific gene lists were selected by unsupervised hierarchical clustering of differentially expressed RNAs between the six different cancer types. Combined first and second ranked classification resulted in an 89% accuracy to classify the tumour types correctly. Finally, the authors assessed whether platelet RNA reflects the molecular profile of the tumour tissue. Selection of biomarker-specific gene lists allowed the discrimination of *KRAS* and *EGFR* mutations, *HER2* amplification and *MET* overexpression compared to wild-type sequences in the platelet RNA profile, which matched the respective tumour DNA analysis.

Thus, this diagnostic platform — based on sequencing RNA obtained from blood platelets and self-learning algorithms — can discriminate patients with cancer from healthy controls, predict the localization of the primary tumour and provide information on the molecular tumour phenotype. Major strengths of the method are that only a minimal amount of blood — as opposed to invasive tissue biopsies — is required and that flexible sample storage conditions will facilitate clinical implementation. Current limitations are the inability to discriminate between different cancer stages and between metastasized versus non-metastasized tumours. Further validation is still required to assess the risk of false positives with increasing sample sizes and to determine whether and how non-cancerous systemic factors (such as inflammation and cardiovascular events) influence the platelet RNA profile and the analytic outcome.

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This article originally appeared in *Nat. Rev. Genet.* (doi:10.1038/nrg4048).

ORIGINAL RESEARCH PAPER Best, M. G. *et al.* RNA-seq of tumor-educated platelets enables blood-based pan-cancer, multiclass and molecular pathway cancer diagnostics. *Cancer Cell* http://dx.doi.org/10.1016/j.ccell.2015.09.018 (2015)