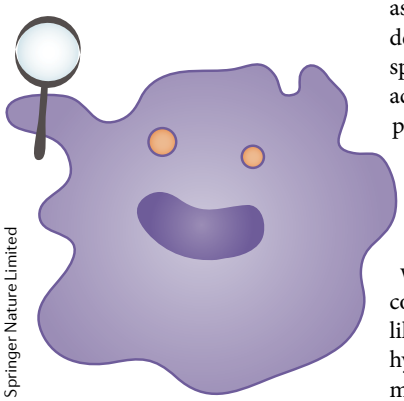
CANCER SCREENING

Searching for a diagnosis

> “Using engineered macrophages as biocompatible diagnostic sensors”

Identifying cancer while it is still localized and treatable holds great promise in reducing cancer morbidity and mortality as well as costs to health-care systems. The past few years have seen rapid development of endogenous biomarker-based tests using circulating tumour cells, circulating cell-free DNA (cfDNA) and cancer exosomes. Yet, these endeavours still lack the sensitivities and specificities required to reliably detect early stage disease. One possible approach to overcome these limitations is the use of probe-based diagnostics that are systemically delivered and selectively activated in the presence of disease. Using engineered macrophages as biocompatible diagnostic sensors, Aalipour et al. have now shown that tumours as small as 25–50 mm^3 can be detected in mice, even with co-occurring inflammation.

Macrophages exist in different activation states that can be broadly classified as pro-inflammatory M1-like and anti-inflammatory M2-like. In solid tumours and a small number of other disease conditions, infiltrating macrophages reprogramme into the M2-like state. Expression of an established M2 macrophage marker, arginase 1 (*Arg1*), was shown to be upregulated in labelled mouse RAW264.7 macrophages intraveneously injected into mice bearing syngeneic 25–50 mm^3 subcutaneous CT26 colorectal tumours, relative to liver-resident or liver-homing macrophages, making it an ideal candidate for a disease-activatable biomarker. Therefore, the authors designed an imageable synthetic reporter by coupling luciferase (a naturally secreted version from *Gaussia princeps*; Gluc) to activation of the *Arg1* promoter to serve as a proxy for tumour detection.



Credit: Lara Crow/ Springer Nature Limited

Following the observation by fluorescence imaging that adoptively transferred macrophages could efficiently home to 25–50 mm^3 tumours, next the authors wanted to determine whether the sensor could detect tumours in vivo through both a blood-based assay and bioluminescence imaging (BLI).

Assaying plasma Gluc levels 24 h after injection of the sensor revealed that tumour volumes in the range of 50–250 mm^3 could be distinguished in mice harbouring subcutaneous CT26 tumours from healthy controls with 100% sensitivity and specificity. In addition, BLI revealed that the activated macrophages co-localized with firefly luciferase-expressing tumours. Even tumour volumes as low as 25–50 mm^3 could be detected with high sensitivity and specificity. Similar findings were achieved with injection of a mouse primary bone marrow-derived macrophage (BMDM) sensor, demonstrating the modularity of this strategy. Importantly, it was noted that necrotic tumours with volumes >1,500 mm^3 could not be discriminated, likely owing to under-perfused hypoxic tumour cores restricting macrophage infiltration.

Recognizing that systemic inflammation is often inextricably linked to cancer, the authors tested whether their sensor would still function effectively under these conditions. In a mouse model of breast cancer metastasis generated by tail vein injection of mouse 4T1 breast cancer cells, the BMDM sensor could differentiate diffuse lung metastatic disease both in the absence and presence of lipopolysaccharide-induced acute lung inflammation.

To directly compare the sensitivity of the macrophage sensor with clinically available cancer biomarkers, the sensor was injected into mice bearing subcutaneous LS174T colorectal tumours, which shed carcinoembryonic antigen (CEA). Plasma CEA levels were measured every third day once the tumours had reached >25 mm^3, with the sensor being administered 24 h before the first plasma sampling. The first measurements, taken 24 h later, revealed that Gluc levels could discriminate mice harbouring tumours (average volume 45 mm^3) from healthy mice while CEA levels could not. Indeed, CEA was only detectable once the tumours had reached on average 137 mm^3. Furthermore, measuring cfDNA concentration could only detect CT26 tumours in mice once these tumours had reached volumes of 1,500–2,000 mm^3.

As the authors rightly point out, this approach currently has shortcomings precluding its advancement into the clinic, such as the possibility that injected M2 macrophages could negatively affect tumour progression, despite the small doses used in the study not leading to altered tumour growth kinetics. Nevertheless, this work presents a strong case for future iterations of immune cell sensors being used as a screening tool to improve patient outcomes.

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