Macrophage Sensors for Early Cancer Detection

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Along the patient continuum of care, the ability to detect cancer at an early stage has an enormous impact on successful drug treatment and patient outcomes. For example, in ovarian cancer, 5-year survival rates rise sharply from ~20% to greater than 90% when lesions are detected at stage I rather than IV, yet only ~15% of patients are detected at the earliest stage due to the poor diagnostic sensitivity of screening. The challenges that limit tumor-shed, or endogenous, biomarkers for early detection are multifaceted. Biomarkers shed from nascent disease sites—which are on the order of a few cubic millimeters in volume but already contain millions of tumor cells—can be diluted by up to 100 000-fold in circulation, making them extremely challenging to detect even with ultrasensitive analytical platforms. Moreover, endogenous biomarkers are secreted by tumor cells at low rates (or not at all) and once in the blood stream biomarkers are rapidly cleared from circulation or are degraded. For biomarkers such as cell-free RNA, their circulation half-lives in blood can be as low as several minutes. Estimates using multicompartment mass transport models have shown that tumor-shed blood biomarkers are at such low concentrations that early tumors may remain undetectable for an entire decade following tumorigenesis (1, 2) based on the detection limits of current biomedical diagnostics. Thus, there remains a stark mismatch between the smallest tumors that can be indicated by blood biomarkers (>2–5 cm) and the size of early stage tumors that would best respond to treatment (<1–5 mm).

The early cancer detection problem is motivating the development of a new class of diagnostics called synthetic biomarkers. The premise is based on the recognition that tumor-shed biomarkers are ultimately limited in diagnostic sensitivity by fundamental mass transport challenges that pose substantial barriers for improvement. Therefore, instead of relying on nature's biomarkers, the route to early cancer detection may require new approaches that focus on synthetic agents that are

precisely engineered to sense, target, and amplify early cancer detection signals in vivo. Recent work has shown that this approach has promise. Systemic delivery of DNA “minicircles” that contain a tumor-specific promoter to drive expression of an exogenous biomarker has been shown to be selectively activated by malignant cells (3), thereby revealing the presence of tumors in mice. By targeting dysregulated protease activity in early tumors, activity-based probes were shown to amplify early cancer detection signals into host urine, providing the ability to discriminate tumors in mice with high diagnostic sensitivity and specificity compared to FDA-approved blood biomarkers (4). In a recent issue of *Nature Biotechnology*, Gambhir and co-authors report the concept of cell-based “immunodiagnostics” by exploiting M2 polarization of macrophages within the tumor microenvironment (TME) as a selective trigger to release a secreted biomarker in adoptively transferred macrophages (5) as a cell-based diagnostic. Without TME cues, these engineered macrophages do not polarize, thereby ensuring detection signals are tumor-specific (Fig. 1).

The authors selected macrophages as a pan-cancer diagnostic cell-sensor based on its high prevalence across different types of cancers. In tumors, macrophages adopt a tumor-associated M2 polarization state that is involved in promoting an immunosuppressive TME. The authors first tested macrophage (both bone-marrow-derived macrophages and RAW264.7) response to immunosuppressive cytokines and tumor-conditioned media, and found a significant upregulation of established M2 genes. The most striking change was found in the expression levels of Arginase 1, which was upregulated by ~600-fold in vitro, and up to 200-fold in vivo when macrophages were adoptively transferred to tumor-bearing mice. Based on these results, the authors designed macrophage sensors by placing the Arginase 1 promoter upstream of a synthetic secreted biomarker, *gaussia luciferase* (Gluc), such that macrophage polarization by the TME would trigger biomarker release into circulating blood for detection.

To confirm that adoptively transferred macrophages infiltrated tumor sites, Gambhir and co-authors showed that macrophages migrated toward tumor conditioned media in transwell assays in vitro, before showing that adoptively transferred macrophages freely infiltrated tumors in mice in a mechanism that was dependent on recruitment chemokines CCL2 and CSF1.

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To test the ability of engineered macrophages to discriminate small tumors, the authors performed adoptive transfer and then monitored the host plasma for secreted Gluc in mouse models of breast cancer lung metastasis, as well as a subcutaneous model of colorectal CT26 tumors. In the lung metastasis model, the authors showed that plasma analysis of Gluc concentrations was able to discriminate metastatic disease from healthy control patients with perfect diagnostic sensitivity and specificity as determined by area-under the receiver-operator-characteristic (AUROC) curves (AUROC = 1.0). The presence of an infiltrate of adoptively transferred macrophages at the sites of metastases was also validated by imaging. To probe the size of the smallest tumors that could be detected using this approach, the authors went on to analyze plasma samples collected from mice bearing colorectal CT26 tumors that ranged in size from 0 to 250 mm³ in volume. Within 24 hours of post-adoptive transfer of macrophages, the authors were able to detect moderately sized tumors (50–250 mm³) with an AUROC of 1.0, and small tumors (<50 mm³) with an AUROC of ~0.85.

To provide supportive evidence that these macrophage sensors were relatively specific for TME activation, the authors went on and adoptively transferred macrophages in models of acute inflammation to quantify potential confounding signals since M2 macrophages are known to be involved in the wound healing process. In two separate models of hind limb or lung inflammation by administration of turpentine oil or lipopolysaccharide, respectively, the authors found that during the acute phase of inflammation, no detected concentrations of their macrophage biomarker were

found in plasma. However, during resolution of the insult when macrophages are known to undergo M2 polarization, the authors detected significant increase in plasma Gluc concentrations. In mice bearing both lung metastasis and challenged with lipopolysaccharide to induce acute inflammation, the authors observed no significant differences in plasma concentrations of Gluc either in the presence or absence of inflammation, suggesting that biomarker signals from their macrophage sensors could discriminate between tumor-associated M2 polarization and the acute phase of inflammation.

Finally, to benchmark their macrophage sensors against clinically used diagnostic standards, the authors went on to compare the diagnostic sensitivity of their approach against a tumor-shed protein biomarker and cell-free DNA (cfDNA). Importantly, in colorectal tumors that aggressively shed the biomarker carcinoembryonic antigen, the authors found that plasma levels of Gluc were able to discriminate tumors earlier and with significantly increased diagnostic sensitivity and specificity compared to carcinoembryonic antigen. Strikingly, in comparisons with the ability of cfDNA shed from tumor cells to indicate disease, CT26 tumors were undetectable by cfDNA analysis until tumors reached 1500–2000 mm³ in volume, while macrophage sensors discriminated significantly smaller tumors (<50 mm³), highlighting the limited diagnostic sensitivity associated with shed blood biomarkers.

The case for developing new cancer diagnostics with synthetic biomarkers is based on the need to circumvent mass transport challenges associated with shed endogenous biomarkers that limit their use for early detection. The innovative approach from Gambhir and co-authors paves the way for new cell-based diagnostics that can actively migrate to tumor sites, respond to environmental cues, and trigger the release of easily detectable synthetic biomarkers. A clear advantage of a cell-based approach is that these living sensors are capable of homing to target disease sites. This is in contrast to systemically administered chemical probes that rely on passive diffusion for delivery, thereby limiting the percentage of injected dose that accumulates in tumors. However, several challenges remain to be addressed with this first study. With cell-based systems, immunogenicity is a concern as it is likely that repeated injections would be required to monitor patients at-risk for early disease or recurrence following remission. Both the secreted biomarker as well as the cell source represent possible sources of new antigens that require mitigation strategies. Moreover, clinical cut-off values for biomarker assays are typically selected to maximize either diagnostic sensitivity or specificity, since there is a trade-off between these two parameters. Here, this study

provides strong evidence that macrophage sensors are sensitive to small tumors ($<50 \text{ mm}^3$); however, substantial sources of biological “noise” are present in the human population (tumor heterogeneity, gender, age) that typically require multivariate biomarker classifiers to differentiate. Thus, this approach will benefit from a multiplexed platform to provide the ability to resolve multiple disease parameters, in addition to TME induction of Arginase 1, and correctly diagnose early cancer from other comorbidities or off-target activation. Last, the ability of macrophage sensors to detect sub- 50-mm^3 tumors is impressive; however, these tumor volumes represent disproportionately larger tumors in humans after allometric scaling. Thus, it is likely that these cell-based sensors would need to be paired with ultrasensitive analytical platforms, such as single-molecule platforms, to further lower the limit of detection of synthetic biomarkers and, thereby, the sizes of tumors that can be detected. This study from Gambhir and co-authors gives traction to an important clinical problem by attacking it in an innovative new way, using the plasticity and homing capabilities of living immune cells as a cancer diagnostic.

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