Diagnostic and intraoperative targeted molecular imaging for pancreatic cancer

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Chapter | 6

On-target probes for early detection

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A cost-effective strategy for early-stage cancer detection would be based on a two-step paradigm1. First, a sensitive and specific blood test screens for individuals in the general or high-risk population who display cancer-specific biomarkers released from tumour cells. Patients with abnormal blood biomarkers then undergo follow-up tests, in which sensitive molecularimaging techniques targeting multiple cancer markers assess the location, size and aggressive state of the putative tumour. Yet such a screening paradigm is not a reality. For tumour detection, current blood biomarkers and imaging probes require high sensitivity to accurately identify small early-stage lesions, as well as high specificity to accurately distinguish benign tissues from malignant transformations [1–3]. However, blood biomarkers such as cancer antigen 125 (CA125) and prostate-specific antigen (PSA) have had limited success in clinical practice because they are also frequently shed by healthy cells in physiological conditions. Even in the pursuit of other types of tumour-specific biomarker, such as patient-specific circulating tumour DNA, more sensitive methodologies and validation are required to provide substantial evidence that these methods can be effective in early cancer screening. On the molecular-imaging front, in which probes are engineered to non-invasively target and visualize a molecular marker indicative of disease in real time, the level of marker expression in cancer cells is often low or heterogeneous, leading to difficulties in distinguishing signals from the tumour and its healthy surrounding tissue, which in turn results in low detection sensitivity. For early cancer detection to succeed using blood-based and imaging approaches, better tumour-to-normal tissue ratios (TNRs) are needed, either by increasing the tumour signal, decreasing the background signal, or both. Two complementary developments reported in Nature Biomedical Engineering now demonstrate how higher TNRs can be achieved with nanoscale probes that are only activated within the tumour microenvironment.

On the one hand, Sangeeta Bhatia and colleagues show that intravenously delivered activity-based nanosensors (ABNs) decorated with tumour-targeting ligands and with peptides cleavable by specific proteases (proteolytic enzymes) overexpressed in the tumour microenvironment can overcome the blood-based detection problems plaguing endogenous secreted biomarkers for early cancer detection [4]. On interaction with specific tumour proteases, the peptide substrates, which are fluorescence-quenched,

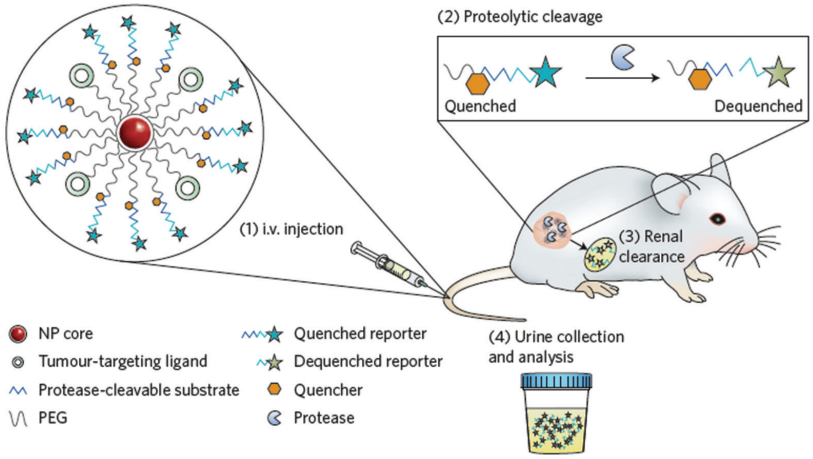

Figure 1. Optimized tumour-activated nanoparticle-based sensors, consisting of a nanoparticle (NP) core decorated with a protease-cleavable substrate and tumour-targeting ligands. Following intravenous (i.v.) injection of the nanoparticles (1), the quenched substrates are cleaved by highly expressed proteases in the tumour microenvironment, which subsequently emit a fluorescence signal (2). The dequenched urinary reporter enters the urine (3) and is detectable via a fluorescence assay (4). Figure adapted from ref. 4, Macmillan Publishers Ltd.

are cleaved and secreted in the urine, where they can be detected by a fluorescence assay (Fig. 1). On the other hand, Xiqun Jiang and colleagues describe the design of a molecular probe, a poly(ethylene glycol)-conjugated iridium (iii) complex (Ir-Im-PEG) that, when activated by two tumour-specific stimuli, results in imaging-signal amplification and thus improved detection sensitivity[5]. The probe responds progressively to subtle differences in extracellular pH and oxygen levels, and distinguishes healthy tissue (which is usually alkaline and normoxic) from tumour tissue (which is acidic and hypoxic).

Bhatia and co-authors, who have championed similar diagnostic nanosystems[6,7], have now optimized the presentation of the nanoparticle-coating substrates on the basis of mathematical modelling of the pharmacokinetics of the ABNs (ref. [6]) to increase the TNR. The authors focused on targeting matrix metalloproteinase 9 (MMP9), as MMP9 mRNA is shown to be upregulated in many human cancers at varying stages of disease[4]. Having previously predicted that a 100-fold improvement in tumour signal was needed to detect sub-5-mm sized tumours, the authors now report the detection of intraperitoneal ovarian tumours as small as 36 mm^3 in mice, outperforming by 2.4-fold the endogenous ovarian

cancer biomarker human epididymis secretory protein 4 (HE4). The authors also show with mouse xenograft models of epithelial cancer and colorectal liver metastasis that the optimized ABNs markedly increase the enzyme-generated diagnostic signal with respect to the signal expected on the basis of ABN accumulation in tumour alone. This suggests that exogenous biomarkers can be optimized to produce urine-based assays with increased sensitivity for the earlier detection of tumours.

Protease-based cancer-imaging probes are making their way from mouse xenograft models to human clinical trials[8]. One question to be answered is how accurately the preclinical results reported by Bhatia and collaborators will translate into early cancer screening in patients. MMP9 is highly expressed in the tumour microenvironment but is also expressed in normal tissue, so activation of ABNs at non-tumour sites may decrease the TNR. Assuming successful nanoparticle delivery and safety, Bhatia and colleagues predict that the ABNs could detect a human serous ovarian cancer 5 months earlier than the clinically relevant biomarker HE4. In reality, extrapolating the behaviour of the ABNs from mice to patients involves the consideration of allometric scaling between species, as well as taking into account biomarker pharmacokinetics, body weight or surface area, tumour vascularity, and possibly other cancerspecific properties. Ultimately, whether ABNs would be sufficiently sensitive and specific for early cancer detection in humans will have to be tested.

Nevertheless, there is a need for better tumour-specific blood-based biomarkers for cancer detection, be them endogenous factors or markers generated from exogenously administered nanoparticles or genetic constructs [9]. Beyond the binary distinction between presence and absence of disease, it is important to identify biomarkers that inform about the state of the malignant tumour. In this context, ABNs or other exogenous biomarkers could be designed to specifically target aggressive and lethal tumours (and to not be activated in benign tumours). Exogenously delivered ABNs, if multiplexed with appropriate enzyme-cleavable substrates and tumour-targeting ligands, may well be a valid strategy to increase the sensitivities and specificities of tumour diagnoses.

Following biomarker-based screening, imaging probes activated at the tumour site provide the possibility to image tissue at high TNRs and therefore validate

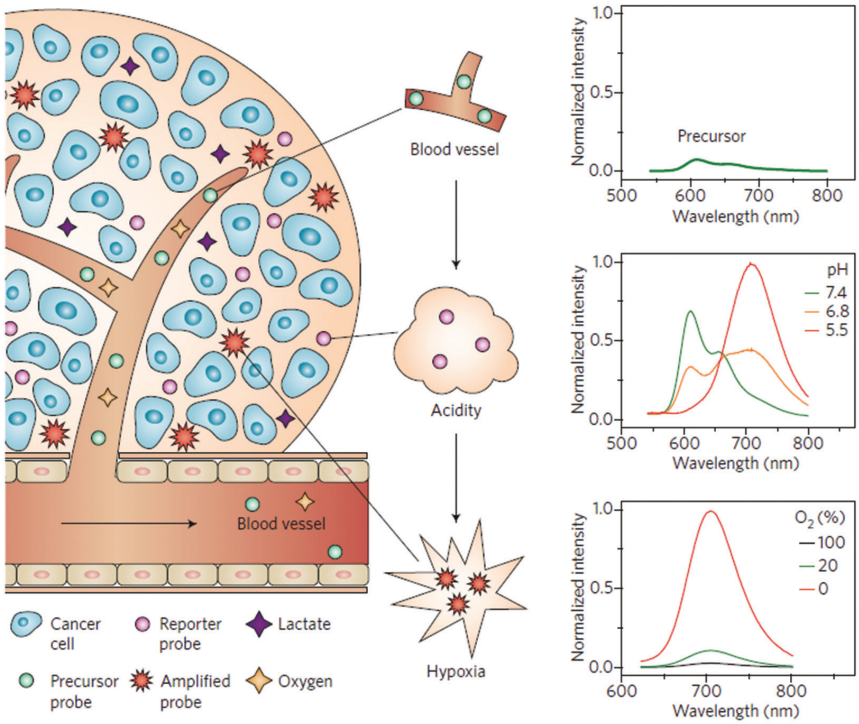

Figure 2. Tumour-activated imaging probe with signal amplification, driven by the conversion of theprobe's precursor form into the reporter form. The intravenously injected precursor probe emits at610 nm. In the acidic tumour microenvironment, the precursor form converts to the reporter form,which emits at 705 nm. At the hypoxic conditions of the tumour microenvironment, the optical signalis amplified. Emission spectra are shown at increasing-pH and decreasing-oxygen conditions. Figure adapted from ref. 5, Macmillan Publishers Ltd.

the potential presence and location of the disease. Jiang and colleagues exploited events occurring in the tumour microenvironment to trigger signal amplification, in contrast to conventional amplification techniques based on chemical or physical modifications of the probe itself. In the acidic tumour environment (pH 6.5–6.9), hydrolysis of the aciditysensitive imine bond in the Ir-Im-PEG complex leads to the conversion of the precursor form of the probe (which emits light at 610 nm) to its reporter form (with emission at 705 nm). If the second stimulus (hypoxia) is also present in the microenvironment, the 705-nm signal is amplified[5] (Fig. 2) The increased sensitivity of this dual-activation approach enabled the detection of tumour nodules as small as 1 mm in diameter. However, one disadvantage of this approach is that optical imaging assays, although useful for localized and intraoperative imaging, may not be ideal for whole-body screening.

In contrast to the behaviour of other microenvironment-responsive probes [10–12], the cleavable Ir-Im-PEG reports coupled events (acidity and hypoxia), and by doing so enhances the detection signal. Indeed, when compared with a previously reported hypoxia-activated iridium macromolecule [13], Ir-Im-PEG achieved roughly a fourfold enhancement in TNR, and improved the detection of small tumour lesions in the liver. The higher TNR of the improved probe was generated by reducing the background signal rather than by increasing the optical signal, so it is possible that the brightness of the probe might not be sufficient for clinical use, for example during real-time surgical resections.

Jiang and co-authors used patient-derived tumour xenograft models and histological analyses of the human tumour microenvironment to validate the Ir-Im-PEG probe. Yet before this imaging approach can be translated to patients, efficacy and dose studies need to be carried out. Nevertheless, the improved sensitivity of Ir-Im-PEG highlights the advantages of enabling signal amplification and of focusing on multiplexing strategies rather than targeting individual markers. The authors' imaging approach could become widely used for the detection of solid tumours, in which subtle changes in acidity and hypoxia are generally present [14,15]. They also showed that such context-sensitive probes might have other applications, for example in wound healing, and that they could pave the way for monitoring the metabolic activity of cells to determine treatment response.

Synthetic nanoparticle systems and optical imaging probes are rarely approved for human use by the US Food and Drug Administration, mainly owing to safety issues, a complex regulatory system, scalability and manufacturing challenges, and a lack of proven benefit in humans. To improve and expedite the clinical translation of new probes for eventual clinical diagnostics, their development should plan for rigorous proof-of-safety and proof-of-benefit studies. These are critical steps in making earlier cancer detection with biomarkers and imaging probes feasible. Although the safety of the probes reported by Bhatia, Jiang and their respective co-authors remains unclear because high dosages might be needed to establish desired effects, it is not too early to state that the detection sensitivity benefits of tumour-activatable probes are promising.

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