Sentinels in the blood: A new diagnostic for pancreatic cancer

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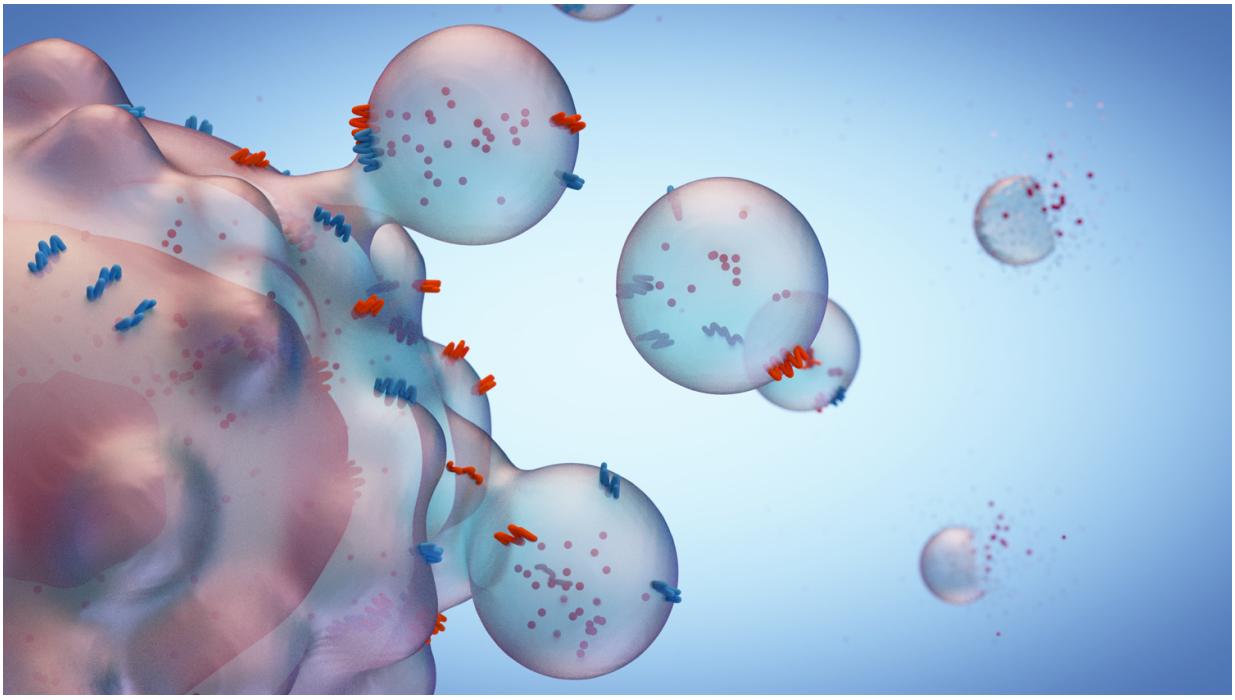


Exosomes are a form of extracellular vesicle (EV) released from most kinds of prokaryotic and eukaryotic cells. Once thought to be mere detritus from cellular metabolism, they are now linked with many critical forms of cell signaling and immune function and play a vital role in a host of diseases, particularly cancer, where they may act to aid metastasis and thwart anti-cancer therapies. Credit: Jason Drees

Despite enormous research strides, detection methods for many diseases

remain cumbersome and expensive, and often uncover illness only at advanced stages, when patient outcomes can be bleak. One such illness is pancreatic cancer, which may display no obvious symptoms in its early stages, yet can develop aggressively. Indeed, according to the American Cancer Society, a staggering 80 percent of those stricken with this form of cancer die within 1 year of diagnosis.

Now, however, Tony Hu, a researcher in the Biodesign Virginia G. Piper Center for Personalized Diagnostics and his colleagues have devised a crafty method to identify pancreatic cancer early in its development. Their technique relies on the sensitive detection of extracellular vesicles (EVs)—tiny bubbles of material emitted from most living cells.

In new research appearing in the advanced online issue of the journal *Nature Biomedical Engineering*, Dr. Hu and his colleagues describe a method to detect EVs derived from tumors that carry a particular surface protein that functions as a telltale marker for pancreatic cancer. The ability to accurately detect this protein, known as EphA2 may allow it to serve as a signpost that could diagnose even the earliest stages of pancreatic cancer.

"Pancreatic cancer is one type of cancer we desperately need an early blood biomarker for," Hu says. Currently, the only cure for pancreatic cancer remains surgical removal of diseased tissue but in many cases, this is not feasible due to the degree of cancer spread at the time of diagnosis. "Other technology has been used for detection, but it doesn't work very well because of the nature of this cancer. It's really hard to capture an early diagnostic signal when there are no symptoms. It's not like breast cancer, where you may feel pain and you can easily check for an abnormal growth."

This research now demonstrates that a platform that uses the interaction between two different nanoparticles to detect tumor-associated EV's can

keenly discriminate between blood samples from patients with pancreatic cancer, pancreatitis—a disease that can share symptoms with pancreatic cancer—and healthy subjects. Further, this technique may ultimately be useful for the rapid and sensitive detection of a range of diseases, based on their unique EV signatures.

Vesicles in focus

EVs are released by both eukaryotic cells (including human cells) and prokaryotic cells, (like bacterial cells, which lack a nucleus or other membrane-bound components). EVs resemble miniature versions of the cells which produce them, though they lack much of the cell's complex machinery.

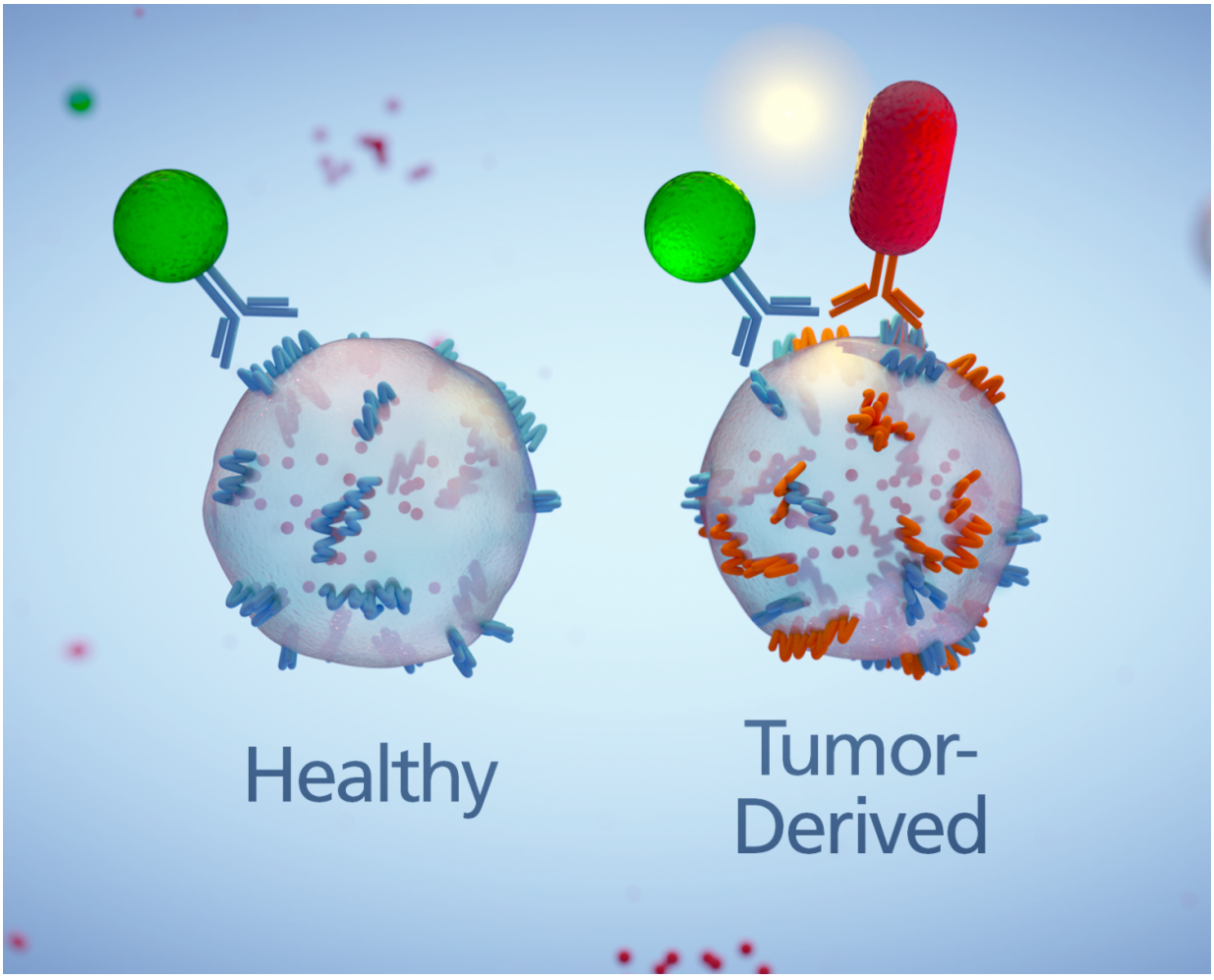
There are a variety EV types, which develop from their parent cells in different ways. The current study examines a class of EVs known as exosomes, which range in size from 50-150 nm. Exosomes are derived from membrane-bound compartments within the cell (known as endosomes) that eventually fuse with the cell's outer membrane to liberate exosomes into the extracellular space.

Once thought to be mere debris from the cell's metabolic activities, EVs are now recognized as vital components with far-flung responsibilities that are only beginning to come to light. EVs form a subtle and sophisticated communications network operating between cells and are highly conserved across species, suggesting their essential role in life processes. Among their activities are the transfer of nucleic acids, proteins and lipids which may trigger physiological and pathological changes, both in parent and target cells. EVs also play crucial roles in innate and adaptive immune responses.

Emblems of health and disease

Research has shown that circulating EVs are significantly elevated in a number of diseases. EV's appear to play an important part in the development and progression of certain cancers, including pancreatic cancer. One apparent function of tumor-derived EVs, once they exit their parent cell, is to migrate to other tissues and modify their surroundings to create an environment (niche) favorable for tumor invasion and growth (metastasis). Like pioneers on a new continent, EVs can thus pave the way for cancer cells to follow in their wake.

There is also evidence that tumor-derived exosomes can help tumor cells develop drug resistance by exporting anti-tumor drugs or neutralizing antibody-based drugs.



The paper describes a new technique for identifying tumor-derived extracellular vesicles (EVs). The method relies on differently shaped nanoparticle probes that refract light at different wavelengths, one spherical (green) and one rod-shaped, (red). One probe identifies a surface protein linked with pancreatic cancer, known as ephA2, and the other identifies a common EV surface protein. Only pancreatic cancer-derived EVs express both proteins and thus bind both nanoparticles to emit a brilliant yellow signal that allows these disease-linked EV's to be easily detected for diagnostic purposes. This method can also usefully track the success of anti-cancer treatment by measuring the abundance of tumor-derived EV's over the course of therapy Credit: Jason Drees

EV's may serve as a useful means of evaluating cancer burden and response to treatment, since levels of tumor-derived EV's in patient blood samples should increase with tumor mass and decrease upon favorable response to cancer therapies, and thus offer a rapid, inexpensive and non-surgical means to examine the changes in the state of a patient's disease.

Identification of tumor-associated EV proteins, such as EphA2, and better understanding of the role of EV's in tumor development and metastasis may thus open a new chapter in cancer diagnosis and treatment monitoring. Given that pancreatic cancer cases are often characterized by high rates of therapy resistance, improved treatment monitoring is urgently needed so that personalized treatments can be quickly modified to improve individual patient outcomes. Further, better understanding of the specific factors that control EV actions to promote cancer development and metastasis may lead to the discovery of new mechanistic targets for cancer treatments that allow custom-tailored therapeutic treatments.

Ray of light

EVs have been isolated from a broad variety of cell types and biological fluids (saliva, urine, blood, breast milk, and seminal, amniotic and nasal and bronchial lavage fluids) making them highly attractive candidates for biomarker development in a variety of conditions. The critical challenge, however, has been separating disease-linked EVs from the diverse array of other EVs circulating in bodily fluids. Researchers lack simple methods for EV analysis, which generally requires time-consuming isolation and purification procedures that are not appropriate for a clinical setting. Further, biomarkers capable of accurately distinguishing tumor-derived EVs have thus far been lacking.

To address these shortcomings, the new method relies on a rapid, nanoparticle-based technique that can quickly identify tumor-derived EVs with minimal preparation.

To do this, small samples of blood (around 1 microliter, less volume than found in a single tear drop), are diluted and applied to a sensor chip coated with antibodies to an EV membrane protein. EVs bound to the chip by this antibody are then mixed with antibody-coated nanoparticles—one green nanosphere and one red nanorod—that recognize a second EV membrane protein and the pancreatic cancer marker EphA2. Only pancreatic cancer-derived EVs bind both nanoparticles, and their close contact on these EVs causes a coupling effect that changes the color and markedly increases the intensity of their refracted light, generating a signal that is easily visible when viewed with a dark field microscope, (see illustration).

In a series of experiments conducted by Dr. Hu and colleagues, this method identified blood samples from pancreatic cancer with high sensitivity, including those with early stage disease, readily distinguishing them from those of pancreatitis patients and healthy

individuals. Further, this method detected alterations in EphA2-EV blood levels in pre- and post-therapy blood samples corresponding to tumor responses to therapy, demonstrating the technique's power to monitor treatment effectiveness.

Although the current study examined samples using light microscopy, the researchers envision a fully automated system capable of performing such assays in the clinic at low cost and high-throughput. Dr. Hu indicates that there is enthusiastic interest for clinical translation of this new diagnostic technology, though he notes that 2-3 years will likely be required for FDA approval.

Promising EV frontier

This approach shows promise for the detection of a broad range of diseases in which EVs may be applied as biomarkers, since it should be possible to customize it by simply replacing one or both nanoparticle probes with EV-specific probes for the disease of interest.

Dr. Hu and colleagues have already demonstrated, albeit in a study with a small number of samples, the validity of this method for detecting active tuberculosis cases. In this study, EVs derived from tuberculosis bacilli were abundantly detected in patient urine samples. These encouraging results open the door for simple, non-invasive TB testing. This is particularly important for patients who cannot produce sputum samples for standard TB tests, and may therefore be required to undergo one or more invasive procedures to obtain a sample that can be used for diagnosis.

EVs are also under study for other disease-specific applications, and the pace of such investigations continues to accelerate. Some researchers are even exploring the ability of EVs to directly serve as drug delivery or therapeutic agents. Thus potential medical applications for EVs, once

disregarded as cellular debris, appear very promising.

More information: *Nature Biomedical Engineering*, nature.com/articles/doi:10.1038/s41551-016-0021

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