"TIPSTer" to the Rescue: A Rapid and Sensitive Method for Detecting Tumor Cells in Pleural Fluid from Lung Cancer Patients

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Introduction. Pleural effusions (PE) is a potentially life-threatening condition afflicting up to 1.5 million people in the U.S. each year. Because the etiology can range from inflammatory/infectious processes to cancer, diagnosing the source of the PE is essential. It is essential to determine whether an effusion is malignant (i.e. due to cancer) as this has profound implications for staging and treatment. Standard pleural fluid cytology is however often insufficient or inaccurate in determining malignant pleural effusions. Here we describe a novel method to identify cancer cells in small samples of pleural fluid.

Methods. We have developed a new diagnostic methodology, which we refer to as Tumor cell Identification in Pleural fluid via Size and TelomERase activity (or TIPSTer), for detecting rare cancer cells in limited amounts of pleural and other bodily fluids. TIPSTer employs a microfluidic-based system (CelSee chip) to isolate cancer cells based on size (cells larger than 8 um) and deformability (cancer cells are relatively more rigid than monocytes) in conjunction with an adenoviral probe that drives GFP expression in the presence of the elevated human telomerase promoter activity that is characteristic of almost all cancer cells. Tumor cells expressing GFP are then identified based on size, shape, and fluorescence intensity.

Results. We first tested and validated TIPSTer in human lung cancer cells in culture and in spiked into control blood. To confirm that the GFP-expressing tumor cells isolated via TIPSTer were of tumor origin, we assessed for thyroid transcription factor 1 (TTF-1) and Napsin A (expressed respectively by well and poorly differentiated lung adenocarcinoma). The usefulness of TTF-1 and Napsin A for this purpose was previously confirmed via western blotting and immunofluorescence staining of positive and negative control cell lines. To simulate a patient PE sample, U251 cells tagged with mCherry were spiked into fluids of human origin and then isolated via a CelSee chip to validate its applicability in capturing tumor cells. Finally, we show that malignant cells can be successfully identified in a PE sample from a patient with non-small cell lung cancer, via the TIPSTer method.

Conclusions. We present promising preclinical and clinical results with this method. We describe future plans for further validation using a greater number of samples, including to identify targetable pre-existing mutation(s) in isolated tumor cells from pleural effusion. This method should ultimately be a useful assay for rapidly assessing if pleural effusion (or other bodily fluids) are due to malignant disease.