

## Poster 15 Non-vesicular cancer-shed particulates: Hidden threat to cancer spread?

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### Background

The molecular basis of cancer metastasis has long been explained by the epithelial-to-mesenchymal transition (EMT) theory. However, the scarcity of EMT-positive circulating tumor cells (CTCs) capable of initiating secondary tumors highlights unresolved gaps in understanding metastatic spread. Malignant tumors actively shed cellular debris and large cell-free particulates whose biological role remains unknown. Here, we investigate the role of non-vesicular cancer-shed particulates (nvCSPs) in direct oncogenic transformation of distant parenchymal cells.

We propose that nvCSPs, actively released by primary tumors, are biologically active and contain payloads with dedifferentiation and oncogenic signals. These nvCSPs while evading immune detection enable de novo transformation of distant parenchymal cells, resulting in the emergence of secondary tumors.

### Methods

We used two types of genetically engineered mouse models for non-small cell lung cancer (NSCLC): a Ras-driven model (SPC-rtTA/TetO-Cre/KRAS-G12D<sup>fl/fl</sup>) and a RAF-driven lineage tracing model (SPC-rtTA/TetO-Cre/CAG-STOP<sup>fl</sup>-DsRed/TetO-HA-CRAF-BxB). We also used fresh-frozen (FF) tumor & normal tissues from HTCR Services GmbH. These models helped us identify and validate the RAS-RAF-MEK-SOX9 signaling axis driving dedifferentiation in metastatic lung tumors. We used confocal microscopy on FF tumor sections to track DsRed-labelled nvCSPs from primary tumors to metastasis sites. We then investigated extracellular biological activity of nvCSP by luciferase-based cell-free promoter activity assays using human NSCLC lines. Lastly, we assessed the presence of biologically active nvCSPs in blood serum from metastatic lung cancer patients.

### Results

Primary lung tumors expressing molecular hallmarks of dedifferentiation actively shed nvCSPs that traverse via lymphatics to the liver, possibly via the hepatic portal vein. These particulates contain oncogenic proteins as well as components for genetic reprogramming. The nvCSPs produced by human metastatic NSCLC lines exhibit promoter-independent protein synthesis activities, suggesting an inherent potential to genetically reprogram distant tissues. Our results provocatively indicate a cell-free mechanism of secondary tumor formation in the liver mediated by nvCSPs, challenging current CTC theory for cancer metastasis.

