Targeting the WASF3 regulatory complex to suppress Metastasis

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The WASF3 gene is involved in actin cytoskeletal reorganization in response to external stimuli from growth factors and cytokines. It is expressed at high levels in metastatic cancers and was part of the gene expression signature defining the claudin-low subgroup of breast cancers. Cells that do not express WASF3 do not metastasize and knock down of WASF3 in metastatic breast cancer cells leads to suppression of invasion in vitro and invasion in vivo. Re-expression of WASF3 in non-metastatic cells increase cell motility and invasion. This strict requirement for WASF3 function in metastatic breast cancer cells suggested targeting this function may provide a means to suppress metastasis. There are currently no small molecule inhibitors of WASF3 function and so we decided to target protein-protein interactions essential for its function. In resting cells, WASF3 is maintained in an auto-inhibited conformation through interaction with a protein complex referred to as the WASF3 regulatory complex (WRC). NCKAP1 and CYFIP1 are important components of the WRC and genetic knockdown of these proteins in metastatic breast cancer cells leads to destabilization of the WASF3 complex. Stimulation of quiescent cells with growth factors activate RAC1/2 which binds to NCKAP1 and relaxes the protein complex to allow phosphoactivation of WASF3. To target the WRC, we developed stapled peptides against alpha helical interaction sites between WASF3 and CYFIP1. Stapled peptides are a new class of therapeutic peptides which show increased stability, resistance to protease degradation, non-immunogenic and are actively transported into cells. Targeting the WASF3-CYFIP1 complex led to loss of phosphoactivation of WASF3 and reduced invasion in vitro. As shown in the crystal structure of the WRC, NCKAP1 does not interact directly with WASF3 but rather binds to CYFIP1. Targeting the CYFIP1-NCKAP1 interaction using stapled peptides led to destabilization of the WRC and loss of invasion of breast cancer cells in vitro. When the two classes of stapled peptides were used in in vivo xenograft studies of MDA231 metastatic cells, compared with vehicle treated animals, metastasis to the lungs and liver was significantly suppressed. Biodistribution studies showed uptake in liver and stomach in early stages and over 72 hours concentrated in tumors. The half-life of the peptides in peripheral blood was ~30 minutes. These studies demonstrate the proof-of-principle that targeting the WRC in breast cancer cell can suppress the metastatic phenotype. Current efforts involve modification of stapled peptides to increase potency, increase retention times in the blood and to increase solubility in formulations that can be delivered intravenously.

Speaker Biography

John K Cowell is Interim Director of the Georgia Cancer Center, Associate Director for basic science and a Professor of Pathology. Prior leadership roles include Director of the Center for Molecular Genetics at the Cleveland Clinic and Chair of the Department of Cancer Genetics at the Rowell Park Cancer Institute in Buffalo, New York. He investigates the molecular genetics of cancer particularly the genetic basis of metastasis in breast and prostate cancer. He is also developing novel therapeutic approaches to the treatment of stem cell leukaemia. His research has been continuously funded by the National Cancer Institute for over 20 years.

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