Isolation Of Tumor Initiating Cells With Metastatic Potential From Human Primary Invasive Ductal Carcinoma.

Carolyn Marsden*†, Mary Jo Wright‡†, Latonya M. Carrier*†, Radhika Pochampally*†▲ and Brian G. Rowan*†. Departments of Structural and Cellular Biology*, Surgery‡, Pharmacology*, Environmental Health Sciences**, and Tulane Cancer Center†, and Center for Gene Therapy▲, Tulane University Health Science Center, New Orleans LA 70112.

Disseminated breast cancer cells may be present at distant sites at the time of primary diagnosis of breast cancer in patients that exhibit no outward signs of clinical metastasis. It is hypothesized that early breast cancer metastasis is initiated by a small population of breast tumor initiating cells (bTICs) within the primary tumor that are inherently resistant to chemotherapy and hormonal therapy. To better understand the biological pathways that permit bTICs to metastasize and to evade current breast cancer therapies, bTICs have been isolated from breast cancer biopsies from patients diagnosed with primary invasive ductal carcinoma. Breast cancer needle biopsies from primary tumors were obtained during the routine care of patients with consent and IRB approval. Core biopsies were mechanically and enzymatically dissociated, yielding a single cell suspension which was subsequently cultured under non-adherent and serum-free conditions to obtain tumorspheres. Injection of about 500 bTICs into the mammary fat pad of female nude mice resulted in tumor formation at the site of injection within two months that was maintained as a small palpable mass for at least six months. H+E staining of sections of the primary tumors revealed complex cellular organization and both microvasculature and macrovasculature. Serial transplantation of the primary tumors from 6/7 samples resulted in tumor formation at the site of injection. Upon primary tumor formation in the mammary fat pad, metastatic human breast cancer cells were detected within the lungs, liver, brain and bone marrow (femur) from 5/7 tumors after six months. Metastatic cells were detected by PCR for an alpha-satellite sequence in the centromeric region in human Chromosome 17, and by in situ hybridization using an human specific Alu DNA oligonucleotide probe. Metastatic bTICs were mostly detected as single cells or small clustering of cells present throughout the metastatic organ. These data demonstrate that cells with the phenotype of "tumor initiating cells" can be isolated from primary biopsies of human invasive ductal carcinoma and cultured in vitro.

Unlike direct heterotransplant tissues from primary tumor biopsies, the majority of bTICs form tumors when injected into immunodeficient mice and further exhibit a highly metastatic phenotype.

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