B8

Methods in the Development of Human Carcinoid Cell Lines

Eric Sceusi[1], Asif Rashid[3], Yunfei Zhou[2], Shaija Samuel[2], Fan Fan[2], Ling Xia[2], Xiang-Cang Ye[2], Jia Lu[2], Federico Tozzi[1], Puja Gaur[1], Patrick Zweidler-McKay[6], Raymond Meyn[5], James Yao[4], and Lee M. Ellis[1, 2]

Departments of [1]Surgical Oncology, [2]Cancer Biology, [3]Pathology, [4]Medical Oncology, [5]Experimental Radiation Oncology and [6]Pediatrics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Background: Research in carcinoid and neuroendocrine tumors (NETs) is limited by the lack of midgut derived cell lines. The existing foregut NET lines, BON and H727, are phenotypically distinct from midgut NETs; highlighting the importance of establishing midgut derived NET cell lines. NETs are difficult to culture and we are developing methods to improve primary NET cell culture.

Methods: Sterile human NET surgical specimens were digested to a single cell suspension and fibroblast depleted using magnetic beads. The NET cells were maintained on collagen I coated plates for 1) culture and 2) immortalization. FACS was performed with a portion of cells using the Aldefluor assay to isolate potential cancer stem cells (CSCs). Sorted cells were grown in serum-free media on low attachment plates to assess sphere formation. Subcutaneous xenografts in nude and NOD/SCID gamma mice using fresh tumor implants and NET cell suspensions were performed.

Results: 1% serum improves primary cultured cell survival compared with 10% serum media. The effect of low serum reverses after approximately one week. Subcutaneous xenografts formed tumors in 5/59 mice over 4-10 months. FACS sorting has identified an ALDH+ population of NET cells, which can form spheres (a characteristic of CSCs) more frequently than ALDH(-) negative cells, which did not proliferate in sphere forming media. NET markers, somatostatin receptor 2 and chromogranin A, were detectable in cultures from a midgut carcinoid liver metastasis at passage 3 and 5, as well as in one mouse xenograft tumor; however both lines failed to grow beyond this point. We are now studying tumor xenografts in more immunodeficient NOD/SCID

gamma mice.

Conclusions: We have developed a system allowing for NET primary culture up to 4 weeks. These techniques are the first step in obtaining cells for in vitro studies. Supported by the The Raymond and Beverly Sackler Foundation.