

Circulating Tumor Cells Failed to Predict Prognosis Following MicroWave Ablation of Oligometastasis in EGFR-mutant Non-Small Cell Lung Cancer Patients

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The process by which the normal breast epithelium turns into breast cancer is poorly understood. The common conception is that the accumulation of random mutations confers a survival advantage which leads to a clonal expansion of populations having the capacity to requisition the micro-environment and to disseminate. The frequent association of high-risk preneoplasia and in situ carcinoma with fully developed invasive cancer suggests that carcinogenesis may be a protracted process involving various cell populations. Normal tissues in the vicinity of breast cancer show signs of clonal epigenetic reprogramming towards reduced differentiation [1], while atypical hyperplasia and ductal carcinoma in situ show numerous genetic [2] and epigenetic changes [3] which characterize invasive breast cancer. We used a comprehensive approach to observe the sequential molecular changes associated with the spontaneous immortalization of cultured benign human mammary epithelial cells (HMEC). Human breast epithelial cells grown in serum-free media will only divide a few dozen times before undergoing a first growth arrest called M0 or selection. The canonical point of view is that this first stasis barrier is mediated by retinoblastoma because the silence of CDKN2A (generally by the methylation of the promoter region) allows to escape from M0 which is followed by a period of rapid cell division called post-selection. The canonical view is that the rapidly dividing post-selection cells begin to accumulate a large number of genomic defects, which ultimately leads to a second period of growth arrest known as M1 or agonescence. This second barrier of stasis has been attributed to a dysfunction of the telomeres

We observed spontaneous immortalization of benign HMEC in a patient with Peutz-Jeghers syndrome. This syndrome is caused by a germline mutation in STK11. The STK11 protein normally controls the activity of members of the AMP-activated protein kinase family (AMPK), ultimately stopping protein synthesis and cell division when energy reserves are insufficient. It also has a role in maintaining cell polarity through the remodeling of the actin cytoskeleton. STK11 is recognized as a tumor suppressor gene. Heterozygous carriers of mutated STK11 develop gastrointestinal polyps and are at considerably increased risk of pancreatic, gastrointestinal, breast, cervical, uterine and testicular cancer.

This research was conducted in accordance with the rules of the Institutional Review Board governing patient-oriented research as well as the HIPAA confidentiality regulations. A fragment of histologically normal breast tissue was obtained from a 24-year-old woman with a clinical diagnosis of Peutz-Jeghers syndrome (PJS) at the time of the partial mastectomy for ductal carcinoma in situ (DCIS). The patient was diagnosed with PJS at the age of three when she underwent resection of the small

intestine for intussusception caused by hamartomatous polyps. At 24 years old, she applied for breast cancer screening. His examination at that time was notable only for the pigmented freckles on his lips. Her family history was positive for breast cancer in her maternal grandmother at age 65 and cancer of the fallopian tubes in her maternal aunt at age 55 (BRCA1 and BRCA2 tested and negative). Her clinical breast exam was normal, but a mammogram showed a 1-cm cluster of microcalcifications in the lei breast, which was diagnosed as DCIS on the needle biopsy. Partial mastectomy returned a small DCIS focus with associated atypical ductal hyperplasia. Sequencing of STK11 in the DNA of peripheral blood mononuclear cells returned STK11 256C

The gene expression profile of early passage HMEC PJ4719 is almost identical to that of the control HMEC (FIG. 2a). The progression towards immortalization is marked by the loss of expression of many genes and the gain of expression of some genes (box in Figure 2). The main component analysis (PCA) identified 4 key components (Figure 2b). The first component shows an excellent separation between the samples and the expected order of progression. The gene set enrichment analysis based on the 130 main genes responsible for PCA (additional table 1) identified more than 100 gene sets with adjusted P values

The progression towards spontaneous immortalization is characterized by a striking global hypo-methylation and a regional hyper-methylation of the CpG islands.

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