ISO-4  Simultaneous detection of proliferation, apoptosis, invasive ability, and cytoskeletal organization in cancer cells by nanodot arrays device

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Objective: Extracellular matrix contains structures from micro-scale down to nano-scale. We hypothesized that cells respond to both micro-structure and nano-structure. The aim is to apply nano-surface device to distinguish cancer cell lines by their invasive potentials. We have fabricated a nanodevice composed of a matrix of nine nanodot arrays with various dot sizes ranging from a flat surface to 10–nm, 50–nm, 100–nm, and 200–nm arrays. Methods: HELA, C33A, ES2, PA–1, TOV–112D, TOV–21G, MG 63, and NIH–3T3 cells were seeded onto the device and cultured for three days. Cell density was measured to examine the proliferation of cells, and scanning electron microscopy (SEM) was performed to assess morphological changes in cells. To evaluate cell adhesion and cytoskeletal reorganization, immunostaining specific to vinculin and actin filaments was performed. Results: The scores for cell proliferation, morphology, distribution of focal adhesions, and cytoskeletal reorganization were obtained. We were able to distinguish between the invasive ability of HELA versus later–staged C33A cells. Ovarian cancer cell lines (ES2, PA–1, TOV–112D, and TOV–21G) also exhibited differential growth parameters that are associated with cell type, grade, and stage. Modulation of the growth of MG63 was also achieved. Conclusion: We have established a platform that can be used to assess multiple parameters of cell growth. A simplified fabrication process ensures mass production and lowers cost. According to our results, the device is capable of distinguishing among cancer cell lines of various stages and also provides basic design parameters for artificial implants. Our device will serve as a convenient and fast tool for tissue engineering and cancer treatment.

ISO-5  Mutation Analyses of Keap1 and Nrf2 Genes in Endometrial Cancer

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[Objective] Nrf2 has been implicated in cancer chemoprevention and elimination of oxidative stress by antioxidants, under the negative regulation of Keap1. However, the discovery of Keap1 and Nrf2 mutations in some cancers casts doubt into the beneficial effects of Nrf2 activation. This study aims to clarify the role of Keap1 and Nrf2 mutations in endometrioid endometrial cancer. [Methods] 105 endometrioid adenocarcinoma samples were used for sequencing of the entire protein-coding region of Keap1 and Nrf2 exon 2, containing Keap1 interacting domains. Nrf2 immunohistochemistry of paraffin-embedded clinical samples and normal endometria was performed. Informed consent was obtained from the patients. The Ethics Committee approved this study. [Results] 9 cases of Keap1 mutations and 3 cases of Nrf2 mutations were identified. Both mutations are mutually exclusive. Mutations were associated neither with the protein expression levels and Nrf2 localization, nor with age, surgical stage, or tumor grade. However, higher Nrf2 protein expression was associated with a lower tumor grade. Nrf2 protein expression is also significantly higher in normal endometria compared with endometrial tumors. [Conclusion] We found a total of 15 novel mutations (one case had two Keap1 mutations) in endometrial cancer. A higher Nrf2 protein expression seems to play a role in cancer prevention.

ISO-6  Impact of deficiency of tumor suppressor genes on tumor development in uterine cervix of IGF–1 transgenic mice

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[Objective] Many human tumors including uterine cervix overexpress insulin-like growth factor (IGF–1) and/or IGF–1 receptor, and serum levels of IGF–1 have been identified as risk factors for cervical cancer. Previously, we generated transgenic (Tg) mice that overexpressed an IGF–1 in epithelial cells driven by the keratin 5 (K5) promoter, and K5.IGF–1 Tg mice developed cervical intraepithelial neoplasia grade 3 (CIN3: ~20% incidence at 1 year). However, no invasive lesions developed in uterine cervix of these mice. In this study, we examined the impact of loss of tumor suppressor genes associated with cervical carcinogenesis on IGF–1 mediated tumor promotion in uterine cervix of K5.IGF–1 Tg mice. [Methods] K5.IGF–1 Tg mice were crossed with p53, PTEN and Bax knockout mice. Uterine cervix of 6-month-old offspring from each genotype were analyzed. [Results] In IGF–1 Tg/PTEN–/– and IGF–1 Tg/Bax–/– mice, the incidence of CIN3 increased to 50% (5/10) and 63% (5/8), respectively, and no invasive lesions developed. In IGF–1 Tg/p53–/– mice, the incidence of cervical neoplasias was 28.5% (4/14), and 2 invasive squamous cell carcinomas developed. [Conclusion] These findings indicate that K5.IGF–1 Tg mice deficient in PTEN and Bax may be more sensitive to tumor promotion of uterine cervix, and that p53 deficiency cooperates with increased IGF–1 signaling to drive tumor progression.