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Detection of CD44$^+$ cancer initiating cells in pleural effusion of a liver cancer patient

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Introduction: Advanced hepatocellular carcinoma (HCC) is always accompanied with multiple lung metastasis which may result in an accumulation of fluid in the pleura. Recently, the concept of cancer-initiating cells in HCC has been established. By studying the cells isolated from the pleural effusion (PE) may provide a model to study the presence of cancer-initiating cells which lead to metastatic disease in HCC. The objective of this case study was to detect a subpopulation of CD44$^+$ cancer-initiating cells in PE.

Methods: PE-derived cells were isolated from PE using ficoll density gradient and primary culture was sustained in DMEM with 30% v/v sterilely filtered PE-fluid to provide the microenvironment for the growth of the isolated cells. Flow cytometry was performed to detect the amount of CD44$^+$ subpopulation. CD44$^+$ and CD44$^-$ cells were then isolated by fluorescent-activated cell sorting (FACS) and spheres formation was performed using 1000 cells/well on an ultra-low attachment plate. CD44$^+$ cells were also subjected to differentiate into osteocytes using the StemPro osteogenesis differentiation kit. PCR analysis was performed to detect the stem cell and liver cell marker of the CD44$^+$ subpopulation.

Results: Flow cytometry analysis demonstrated that over 90% of the cells isolated were CD44$^+$ cells. PE-derived cells were able to be expanded in culture for several weeks. CD44$^+$ but not CD44$^-$ cells were able to form spheres in vitro. CD44$^+$ cells were also able to be differentiated into osteocytes with positive staining of calcium accumulation using Alizarin Red S staining. PCR analysis demonstrated the expression of markers for stem cell (Lin28, Oct4, Nanog, Sox2 and Msi1) and liver cell (alpha-fetoprotein, albumin and asiaglycoprotein receptor).

Conclusion: This is a case study demonstrating the presence of cancer-initiating cells in PE by detection of CD44$^+$ cells. The isolation of PE-derived cells might provide a model for subsequent molecular and cellular analyses, which allows the study of cancer-initiating cells in metastatic HCC patients and provides a direction for the preclinical development of rational therapeutics.

Plasma pigment epithelium-derived factor as a mediator of insulin resistance associated with estrogen deficiency

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Objective: Pigment epithelium-derived factor (PEDF), a serine protease inhibitor secreted from adipose tissue and present at high concentrations in the circulation, has been found in mice to play a pathogenic role in obesity-induced insulin resistance and metabolic dysfunctions. Serum PEDF levels are lower in women and treatment of cultured human ovarian surface epithelial cells with 17β-estradiol inhibited the expression of PEDF at a transcriptional level. To further delineate the relationship between estrogen and PEDF, we investigated the change in PEDF levels in pre-menopausal women following bilateral salpingo-oophorectomy (BSO).

Methods: We measured body mass index (BMI), waist circumference (WC), percentage body fat, plasma PEDF and estradiol levels in a group of pre-menopausal women before and after BSO for benign gynaecological conditions. Repeated measure ANOVA was used to analyse the changes (Δ) in PEDF, estradiol, homeostatic model assessment of insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index (QUICKI) following BSO.

Results: Twenty-one pre-menopausal women (age, 49.57±3.54 years) were recruited. The mean duration between pre- and post-operative assessment was 4.01±0.72 months. BMI was marginally reduced in the postoperative period (25.24±3.98 kg/m^2 preop vs 24.80±4.34; P=0.048). However, no significant difference between WC or percentage body fat was observed. The marked reduction in plasma estradiol levels postoperatively (225 [109-418] pmol/L preop vs 32 [19-50]; P<0.001) was accompanied by an increase in plasma PEDF levels (7.66±1.61 ng/mL preop vs 8.77±1.53; P=0.001, after controlling for change in BMI). An inverse relationship was found between Δestradiol and ΔPEDF (r= –0.497; P=0.022). There was also a significant increase in insulin resistance postoperatively, as indicated by HOMA-IR (1.06 [0.73-1.93] preop vs 1.63 [1.06-2.26]; P=0.002) and QUICKI (0.38±0.04 preop vs 0.36±0.03; P=0.006). ΔHOMA-IR and ΔQUICKI became insignificant (P=0.101 and 0.141 respectively) after adjusting for ΔPEDF.

Conclusions: We have demonstrated that the reduction in plasma estradiol levels after BSO was significantly associated with an increase in plasma PEDF levels and insulin resistance in a group of pre-menopausal women. The loss of statistical significance in ΔHOMA-IR and ΔQUICKI after adjusting for ΔPEDF suggests that PEDF may play a role in mediating insulin resistance in these women with surgical menopause, and its expression is likely regulated by estradiol, as evidenced by the correlation between Δestradiol and ΔPEDF.