compared. We focused on the effect of the two agents in the migration capacity of the human breast cancer cells. There are two mechanisms of cell migration, the single cell migration that occurs during metastasis and collective single migration that occurs during invasion.
Materials and Methods: Two hormone-dependent human breast cancer cell lines MCF-7 and T47D were used. Cells were treated with fulvestrant, tamoxifen, and the two metabolites of tamoxifen, endoxifen and 4-OH-tamoxifen. The proliferation of the cells was determined using the MTT assay. The migration capacity was evaluated using the boyden chamber and the scratch wound assays.
Results: The tested agents inhibited the proliferation of both cell lines after stimulation of ER by estradiol with a dose dependent manner. Then single cell migration was studied using boyden chamber assay and it was found that fulvestrant was superior than tamoxifen and its metabolites. The scratch wound assay reflects collective cell migration and it was found that the inhibitory effect of tamoxifen and its metabolites was greater than fulvestrant.
Conclusions: The results of the current study confirm the antitumour effect of fulvestrant and tamoxifen in hormone dependent breast cancer cells. However, it is the first time that the two agents are compared according to their effect on breast cancer cell migration. The results demonstrate that there are more than one ways for breast cancer cell migration and the two agents affect different mechanisms. Further research on the pathways that control the different mechanisms of cell migration are necessary and are ongoing.

1066 POSTER

Interleukin-6 Increases Metastasis Formation Through Mobilization of Immature Myeloid Cells to the Pre-metastatic Niche

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Interleukin-6 (IL-6) is a key inflammatory cytokine that has been shown to link immune cell function with cancer progression. IL-6 produced by tumour cells is associated with increased tumour cell proliferation and regulation of the immune system with reduction of mature dendritic cells through activation of STAT3. However, the systemic effects of host-derived IL-6 have not been delineated. More specifically, the role of this cytokine in the modulation of maturation and recruitment of immature myeloid bone marrow-derived cells (BMDCs) and formation of the pre-metastatic niche has not yet been addressed. To demonstrate the role of IL-6 in this process, wild-type (WT) and IL-6 knock-out (KO) C57Bl/6 mice were injected either subcutaneously with B16 melanoma cells or orthotopically with EO771 breast adenocarcinoma cells. For both tumour models, there remained no differences in primary tumour growth, however, a decrease in lung metastasis was observed in IL-6 mice as compared to WT mice at 3 and 5 weeks respectively post tumour injection. By immunohistochemistry, there was an increase of pSTAT3+ cells and CD45+ cells in the pre-metastatic lung in WT as compared to IL-6 mice. In further analysis of this cell population by flow cytometry, we observed an elevation of CD11b+Gr1+ cells in the lungs and in the circulation of WT mice compared with IL6 KO mice. To confirm that STAT3 activation promotes the mobilization of BMDCs, we used a conditional STAT3 overexpression transgenic mouse model. After 5 days of doxycycline treatment, we observed an increase of recruited STAT3+ cells and CD45+ cells to the lungs and of CD11b+Gr1+ progenitor cells in the circulation and to the lungs. When these mice were crossed with IL-6 KO mice, there was a complete reversion of this phenotype. Our results suggest that host IL-6 promotes metastatic growth in the lungs through mobilization and recruitment of immature myeloid cells to the pre-metastatic lungs, and that this mobilization and recruitment is dependent upon STAT3 activation.

1067 POSTER

Study of the Effects of Dietary Flavonoids, Luteolin and Quercetin on the Reversal of Epithelial-mesenchymal Transition in A431 Epidermal Cancer Cells

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In our earlier studies, we documented the antiproliferative and anti-metastasis effects of luteolin and quercetin. Appreciation of the diverse anticancer activities exerted by these two flavonoids prompted us to evaluate their impact on events such as tumour progression and invasion. Highly invasive A431-III cells derived from parental A431-P cells, were isolated via three successive passages through Boyden chamber with matrigel-coated membrane support (*Anticancer Research.* 28: 2109–2120, 2008). The greater invasion potential exhibited by A431-III cells was owing to increased ability for spreading, migration and enhanced MMPs activity. Comparison of tumour progression events evoked by A431-P cells with those manifested by A431-III cells could emerge as a useful strategy for evaluating of EMT. These cells might afford a reliable model for the evaluation of tumour metastasis events (*Cancer Science*, 102: 815–827, 2011). Employing this approach, we evaluated the effects of luteolin and quercetin, with the A431-P/A431-III EMT model. These flavonoids reversed cadherin switching, downregulated EMT markers and nullified the invasion ability of A431-III cells. Overexpression of MMP-9 resulted in inducing EMT in A431-P cells and this could be reversed by treating with luteolin or quercetin. Co-treatment of A431-P and A431-III cells with EGF plus luteolin or quercetin caused these cells to become more epithelial-like in morphology, led to a fall in the levels of EGF-induced markers of EMT and the restoration of cell-cell junctions. E-cadherin was decreased by EGF, but increased by luteolin and quercetin. Our emerging data from the present investigations suggest that luteolin and quercetin are potentially beneficial agents to intercept and prevent EMT occurrence in epidermal carcinoma cells and manifest activities relevant to the attenuation of tumour progression in A431-III cells. In conclusion, luteolin and quercetin may have inherent potential to function as chemopreventive and anti-neoplastic agents in abating tumour progression through the reversal of EMT in cancer.

1068 POSTER

Preconditioned Monocytic Endothelial Progenitor Cells Reduce Formation of Melanoma Metastases Through SPARC-driven Cell-cell Interactions

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Background: Tumour progression is associated with the release of signaling substances from the primary tumour into the bloodstream. Tumour-derived cytokines are known to promote the mobilization and the recruitment of cells from the bone marrow, including endothelial progenitor cells (EPC). Here, we examined whether such paracrine influence could also influence the capacity of EPC to interfere with circulating metastatic cells.
Materials and Methods: We examined the effects of preconditioned EPC by the tumour cell secretome on metastases formation using mouse EPC and B16 melanoma cells as well as human EPC and a highly metastatic human breast cancer cell line. Luciferase expression by tumour cells was used to track metastases while 2D-DIGE proteomic analysis and gene silencing strategies were used to identify relevant changes in the EPC phenotype.
Results: We injected EPC pre-stimulated by tumour conditioned medium (CM-EPC) and B16 melanoma cells to mice. A net decrease in metastases spreading (vs non-stimulated EPC) led us to carry out a 2D-DIGE proteomic study to identify possible mediators of EPC-driven protection. Among 33 proteins exhibiting significant changes in expression, osteonectin/SPARC (Secreted Protein, Acidic and Rich in Cysteine) presented the highest induction after EPC exposure to CM. We then showed that contrary to control EPC, SPARC-silenced EPC were not able to reduce the extent of metastases when injected with B16 melanoma cells. Using adhesion tests and the hanging drop assay, we further documented that cell-cell interactions between CM-EPC and melanoma cells were promoted in a SPARC-dependent manner. This interaction led to the engulfment of melanoma cells by CM-EPC, a process prevented by SPARC silencing and mimicked by recombinant SPARC. Finally, we showed that contrary to melanoma cells, the pro-metastatic human breast cancer cell line MDA-MB231-D3H2 reduced SPARC expression in human EPC and stimulated metastases spreading.
Conclusions: Our findings unravel the influence of tumour cells on the EPC phenotype through a SPARC-driven accentuation of macrophagic capacity associated with limitations to metastatic spread, thereby adding a new layer of complexity in the role of so-called endothelial progenitor cells in tumour progression.