

### Introduction

Previous results from our laboratory have demonstrated that breast cancer metastastic potential is inversely related to expression of the gap junction protein connexin (Cx) 43 (1). Furthermore, it has been suggested that heterotypic gap junctional communication between breast cancer cells and osteoblastic cells contributes to breast cancer cell metastasis (2). To further address this issue we examined the expression of matrix metalloproteinase-1 (MMP-1), an enzyme associated with increased metastatic potential, in breast cancer cell lines with altered Cx43 expression. We also examined the effect of co-culture with osteoblastic cells on breast cancer cell MMP-1 expression.

### Methods

MMP-1 levels released into culture media were determined using a commercially available ELISA kit and steady state levels of MMP-1 mRNA determined by semi-quantitative RT-PCR. The cell lines examined included MDA-MB-435 (435), a metastatic breast cancer cell line that does not express Cx43; 435/BRMS1, 435 cells expressing the metastasis suppressing gene BRMS1 and in which Cx43 can be detected; 435/Cx43-1, 6, and 8, three clones of 435 genetically engineered to express Cx43; 435/HY, control transfectant for 435/Cx43 cells and hFOB, a human fetal osteoblastic cell line that expresses abundant Cx43. We examined MMP expression in these cell lines in monoculture and in co-culture with hFOB cells. In some co-culture experiments the different cell types were in physical contact with one another while in some experiments a transwell insert with a microporous membrane filter bottom was used to culture the different cell types within the same media but not in physical contact with one another. To determine the role of gap junctional intercellular communication (GJIC) in any effect of co-culture on MMP-1, some experiments with cells in physical contact were completed in the presence of the gap junction blocker 18a glycyrrhetinic acid (AGA). Differences in MMP-1 protein levels were assessed by ANOVA and a Student-Newman-Keuls post hoc test.

### Results

We were unable to detect MMP-1 by RT-PCR (figure 1) or ELISA (figure 2) in osteoblastic hFOB cells in monoculture. Metastatic 435 cells expressed abundant MMP-1 mRNA and protein whereas metastasis suppressed 435/BRMS1 cells, but not the control for BRMS1 transfection (not shown), demonstrate dramatically reduced MMP-1 mRNA and protein (p<0.05 vs. 435/pvc). Three 435/Cx43-1 clones (1, 6 and 8) released significantly less MMP-1 than either 435 cells or the control. RT-PCR of 435/Cx43-1 clone 1 (figure 1) revealed that MMP-1 RNA were also reduced relative to 435 cells. We next examined MMP-1 in breast cancer cells in co-culture with hFOB cells. Because MMP-1 is undetectable in hFOB cells, MMP-1 in co-culture was presumed to be released by breast cancer cells. All of the breast cancer cell lines examined were displayed both reduced MMP-1 protein (figure 2) and mRNA (not shown) when co-cultured with hFOB cells relative to when in monoculture. Breast cancer cell numbers were similar in monoculture and co-culture suggesting that different cell numbers did not contribute to the results. Interestingly, when these experiments were repeated with breast cancer cells and hFOB cells in co-culture, but not in physical contact, MMP-1 levels were not reduced relative to the same breast cancer cell line in monoculture. Additionally, hFOB conditioned media did not affect breast cancer cell MMP-1 levels. Taken together these results suggest that physical contact and perhaps GJIC contributes to the effect of hFOB co-culture. To examine this possibility we repeated co-culture experiments in which hFOB and breast cancer cells were in physical contact, in the presence of AGA. Once again co-culture with physical contact with hFOB cells in the absence of AGA reduced MMP-1 levels in all breast cancer cell lines, surprisingly, however, this was also the case in the presence of the gap junction blocker AGA, suggesting that physical contact but not GJIC is necessary for the effect of co-culture with hFOB cells to reduce MMP-1 expression in breast cancer cells.

### Discussion

Our findings suggest that restoring Cx43 expression in metastatic 435 cells, which do not normally express Cx43, reduces the expression of MMP-1, an enzyme associated with increased metastatic potential. We have previously demonstrated a similar relationship between Cx43 expression and osteopontin, another protein the expression of which is associated with metastasis. Taken together these results strongly support the concept that the absence of Cx43 contributes to breast cancer cell metastatic potential. However, restoring Cx43 expression in 435 cells did not reduce MMP-1 levels to the same degree as did expressing the metastasis suppressing gene BRMS1 suggesting that the reduced metastatic potential imparted by BRMS1 expression involves other mechanisms in addition to altered Cx43 expression. We also found that co-culturing with hFOB cells reduced MMP-1 levels in breast cancer cells, regardless of their Cx43 status, in a manner dependent on physical contact but not GJIC. This finding suggests the intriguing possibility that by reducing MMP-1 levels in breast cancer cells osteoblastic cells function to protect the bone microenvironment from invading cancer cells. Thus, the ability of breast cancer cells to metastasize to varying degrees may be a function of osteoblastic phenotypic characteristics in addition to breast cancer cell phenotypic characteristics.

### Figure 1

RT-PCR analysis of MMP-1 mRNA. No MMP-1 mRNA was detected in hFOB or 435/BRMS-1. MMP-1 was detectable in 435/Cx43+ but at reduced levels relative to 435.

### Figure 2

MMP-1 Levels in media from breast cancer cells and hFOB cells in monoculture and in breast cancer/hFOB cell co-culture. In monoculture (white bars) no MMP-1 was detected in 435/BRMS-1 and hFOB cells. MMP-1 in 435/Cx43-1, 6, and 8 was significantly decreased relative to vector controls (435/HY 5 and 6). All breast cancer cells released decreased levels of MMP-1 when in co-culture with hFOB (black bars). * Significantly different from 435/HY 5 and 6. + Significantly different from monoculture. Values are mean ± SE, P<0.05 n=6.

### References

1. Saunders et al Cancer Research 2001; 61, 1765-1767
2. Li et al 2002; Orthopaedic Research Society 48th Annual Meeting 186

This work was supported by grants from NIH CA90991 and Penn State University Cancer Center (2002)