Transcriptomic signature of cell lines isolated from canine mammary adenocarcinoma metastases to lungs

M. Król, J. Polañska, K.M. Pawłowski, P. Turowski, J. Skierski, A. Majewska, M. Ugorski, R.E. Morty, T. Motyl

Abstract. Metastasis is a final step in the progression of mammary gland cancer, usually leading to death. Potentially, a molecular signature of metastasis can be defined via comparison of primary tumors with their metastases. Currently, there is no data in the literature regarding the molecular portrait of metastases in dogs and only few reports regarding human cancer. This is the first report describing the transcriptomic signature of canine cancer metastatic cells. Two adenocarcinoma cell lines isolated from the canine mammary gland (CMT-W1 and CMT-W2) were compared with cell lines isolated from their lung metastases (CMT-W1M and CMT-W2M) with regards to the following cytometric parameters: cell cycle, ploidy, Bcl-2 expression, susceptibility to induced apoptosis, and transcriptomic profile. Cytometric analyses revealed significant differences in cell cycle and antiapoptotic potential between the examined cells. Using oligonucleotide microarrays, we found 104 up-regulated genes in the metastatic cell line CMT-W1M and 21 up-regulated genes in the primary CMT-W1 cell line. We also found 83 up-regulated genes in the CMT-W2M cell line and only 21 up-regulated genes in the CMT-W2 cell line. Among the up-regulated genes in both metastatic cell lines, we found 15 common genes. These differently expressed genes are involved mainly in signal transduction, cell structure and motility, nucleic acid metabolism, developmental processing, and apoptosis (GHSR, RASSF1, ARF1GAP, WDR74, SMOC2, SFRP4, DIAPH1, FSCN1, ALX4, SNX15, PLD2, WNT7B, POU6F2, NKG7, and POLR2F). Seven of them are involved in a cellular pathway dependent on ghrelin via growth hormone secretagogue receptor (GHSR). Our results suggest that this pathway may be essential for mammary cancer cells to have a metastatic potential.

Keywords: canine mammary cancer, lung metastasis, microarray, transcriptomics.

Introduction

Mammary cancer is the most common malignant neoplasm in the bitch (Dorn et al. 1968). The annual incidence in dogs is 3 times higher than in humans (MacEwen 1990). In both species, metastases of mammary gland tumor to lungs or lymph nodes are the main problem. Metastasis is a final step in the progression of mammary gland cancer, usually leading to death. The precise nature of the metastases is still not clearly defined. It has been suggested that metastases may arise from a tumor progressing to a pre-malignant state without any significant changes in gene expression (Hynes 2003). Another hypothesis is that a metastasis is an escape of carcinoma cells from the primary tumor (via lymphatic and blood vessels) to the target organ (Wang et al. 2002). Fox and co-
workers (1990) suggested that metastatic clones are derived from one or more initial cells of the primary tumor. Metastasis is also regarded as a genetic evolution of the primary cancer, which suggests that the gene expression pattern of a metastatic tumor is different from that of the primary tumor.

Ramaswamy et al. (2003) suggested that the molecular signature of metastasis, also termed the metastatic gene signature, could be detected by comparison of primary tumors with their metastases. Kang et al. (2003) defined a “bone metastasis signature”, by identifying genes responsible for metastasis in bones. They found 5 overexpressed genes that enhanced metastasis in bones. Individual overexpression of any individual gene was insufficient to increase the metastatic ability of the cancer, but overexpression of some genes at the same time highly elevated the metastatic potential of the primary tumor. Kakiuchi et al. (2003) showed different patterns of gene expression of human small lung cell cancer in each site of metastasis (lung, liver, kidney, bone), suggesting that the metastatic signature is different in each site.

There is no published data regarding the transcriptomic portrait of metastases in dogs. This is the first study undertaken to identify genes responsible for metastasis in bones. They found 5 overexpressed genes that enhanced metastasis in bones. Individual overexpression of any individual gene was insufficient to increase the metastatic ability of the cancer, but overexpression of some genes at the same time highly elevated the metastatic potential of the primary tumor. Kakiuchi et al. (2003) showed different patterns of gene expression of human small lung cell cancer in each site of metastasis (lung, liver, kidney, bone), suggesting that the metastatic signature is different in each site.

Materials and methods

Cell lines and cell culture

The cell lines used in this study were isolated from mammary adenocarcinomas (CMT-W1 and CMT-W2) or their metastases to lungs (CMT-W1M and CMT-W2M, respectively) of 2 bitches. The epithelial origin of all these cells was confirmed by immunohistochemical evaluation. The expression of cytokeratin, vimentin, muscle actin, S100, and p63 proteins was measured (data not shown). The pathological and immunohistochemical examination was based on WHO classification.

Cells were cultured in optimal conditions: RPMI-1640 medium enriched with 10% (v/v) heat-inactivated FBS, penicillin-streptomycin (50 iU/mL), and fungizone (2.5 mg mL⁻¹), in an atmosphere of 5% CO₂ and 95% humidified air, at 37°C, and routinely subcultured every second day. The detailed methods of canine mammary cancer cell culturing were as described in our previous publication (Król et al. 2009).

Induction of apoptosis and immunofluorescence staining for cytometry

The test of cell culture exposure to camptothecin (CPT) was applied to examine cell susceptibility to apoptosis. CPT is an inhibitor of DNA topoisomerase I and is used as an anticancer drug. This method and CPT doses were described in our previous study (Król et al. 2009).

Exponentially growing cells were seeded on Lab-Tek 4-chamber culture slides and cultured for 24 h. For apoptosis induction the medium was then removed and replaced with a medium containing 0.3 μg mL⁻¹ CPT for 1, 6, 9 and 12 h (cells cultured in 10% FBS medium without CPT were used as a control; 4 replicates were performed).

Cells were fixed in 0.25% formaldehyde for 15 min, washed twice with PBS, suspended in ice-cold 70% methanol, and maintained at 4°C for 30 min. Finally, methanol was aspirated and samples were stored at −80°C until staining. For antiapoptotic potential measurement, cells were washed twice with PBS, and incubated in the dark for 30 min with fluorescein isothiocyanate (FITC)-conjugated anti-Bcl-2 antibodies diluted 1:100 with PBS. The Bcl-2 protein is commonly known as the main antiapoptotic factor, and its overexpression in cancer cells ensures resistance to chemotherapy. The Bcl-2 content was compared in all examined cancer cell lines, by using FITC-conjugated anti-Bcl-2 antibody and calculating mean Bcl-2-related fluorescence in a...