EXPRESSION OF THE MATRIX Gla PROTEIN IN UROGENITAL MALIGNANCIES

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Matrix Gla protein (MGP), a vitamin-K-dependent protein which is synthesized in a variety of tissues such as lung, heart, kidney, cartilage and bone. The function of MGP in these tissues is unclear. We have previously reported elevated MGP mRNA levels in a breast-cancer cell line, 600PEI, as compared to normal breast epithelium. Here we describe high MGP expression in primary renal-cell carcinomas, prostate carcinomas and testicular germ-cell tumors, as determined by Northern analysis. MGP was over-expressed in 21 out of 28 patients with renal-cell carcinoma, and in 16 out of 29 patients with testicular germ-cell tumors, as compared to matched normal tissues. For the renal-cell carcinomas, a statistically significant inverse correlation was observed between the level of MGP expression and tumor size, lymph-node metastasis and tumor grade. MGP was also highly expressed in 13 primary prostatic carcinomas as compared to prostate cell lines derived from metastatic tumors, and to lymph-node metastasis. Our findings indicate that the loss of MGP expression may be associated with tumor progression and metastasis.

Primary breast carcinomas were obtained from the tumor bank at the Lineberger Comprehensive Cancer Center. The breast-cancer cell line 600PEI was kindly provided by Dr. H.S. Smith, University of California at San Francisco.

DNA probe

The probe was a 451-bp fragment PCR-radiolabeled by a method previously described (Jansen and Ledley, 1989) from a cDNA clone (pM3) of the human matrix Gla protein gene. The clone pM3 was constructed by subcloning a 561-bp EcoRI-HinII restriction fragment of the EA1 clone (Chen et al., 1990) into a pMAMneo vector.

Northern analysis

Total RNA was extracted from tumors in the presence of 4 M guanidinium isothiocyanate (Chirgwin et al., 1979). The RNA was fractionated by electrophoresis through 1.2% agarose gels containing 2.2 M formaldehyde (Seed, 1982). After electrophoresis, the gels were washed with distilled water for 2 hr to remove formaldehyde, and RNA was subsequently transferred onto Zeta-Probe nylon membrane (Bio-Rad, Richmond, CA), without further pre-treatment (Thomas, 1980). Hybridization was performed in 20 mM sodium phosphate buffer, pH 6.7, containing 10% dextran sulfate, 50% formamide, 1% SDS, 0.5% powdered non-fat milk, 100 µg/ml salmon sperm DNA, and 5 x SSC (20 x SSC = 0.3 M trisodium citrate buffer, pH 7.0, 3M NaCl).

The blots were normalized for RNA loading either with α-actin or by ethidium bromide staining. The autoradiographs were submitted to densitometric scanning, and MGP values were expressed as optical density ratios of MGP/normalizer.

Statistical analysis

The Student’s t-test analyses were performed using the Statview® Program (Brain Power, Calabas, CA).

RESULTS

Matrix Gla protein expression in renal, prostatic and testicular tumors, was evaluated by Northern analysis. The MGP mRNA levels were estimated in relation to the 600PEI breast-cancer cell line (Chen et al., 1990) which showed the highest MGP expression and was arbitrarily considered as 100%.

MGP over-expression in renal-cell carcinomas and testicular germ-cell tumors

The MGP expression pattern for 28 renal-cell carcinoma specimens (RCC) matched with their normal kidney tissue, revealed that 75% (21 samples) over-expressed MGP (over-expression defined as a ratio of MGP mRNA of tumor/normal

Material and Methods

Tumor samples

Primary prostate carcinomas and prostate cell lines were obtained from Duke University (Durham, NC).

Primary renal and testicular tumors were obtained from the Urologische Klinik, Heinrich-Heine-Universität (Düsseldorf, Germany), and Urologische Klinik, Bundeswehrkrankenhaus (Hamburg, Germany). Normal kidney and testicular tissues were taken from areas of the same organ as far from the tumor as possible. Both normal and tumor tissues were confirmed histologically by a single pathologist.

Abbreviations: Matrix Gla protein, MGP; Gla, γ-carboxyglutamic acid.

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Matrix Gla Protein in Tumor Progression

Inverse correlation between MGP expression and clinical status in renal-cell carcinomas

The variability in the transcription levels of MGP from patient to patient suggested that the level of MGP expression may be associated with particular clinical parameters. Using Student’s t-test analysis in renal carcinomas, we observed an inverse correlation between MGP expression ratios of tumor/normal tissues and clinical stage in terms of tumor size (T), and lymph-node metastasis (N). The patients were divided into groups of small vs. large tumor sizes (T1+ vs. T3, respectively), as well as without vs. with lymph-node metastasis (N0 vs. N1, respectively). As shown in Figure 2, the T1+ group exhibited a mean MGP value twice as high as the value observed for the T3 group (mean MGP of T1+ = 2.00, mean MGP of T3 = 1.06, t = -1.78, 0.025 < p ≤ 0.05). A similar observation was made between the N groups (mean MGP N0 = 1.95, mean MGP N1 = 1.02, t = -2.19, 0.01 < p ≤ 0.025). An inverse correlation was also found between MGP ratios of tumor/normal tissues and tumor grade, “G” (G I + II vs. G III; mean MGP of G I + II = 1.85, mean MGP of G III = 0.69, t = -2.10, 0.01 < p ≤ 0.025; data not shown). For the patients with testicular tumors, no statistically significant correlation between MGP expression and tumor stage or grade was found.

High MGP expression in prostatic carcinomas

Northern analysis performed on 13 primary prostatic carcinomas showed high expression of MGP (Fig. 3a,b) as compared with absence of expression in 3 prostatic cell lines derived from metastatic tumors (ILN, DUPRO, DU145), and a xenograft (DU9479, bone metastasis of a prostate tumor). In the 3 prostatic cell lines (ILN, DUPRO, DU145), MGP mRNA could not be detected even by reverse polymerase chain reaction (data not shown). Furthermore, in one patient in whom both primary tumor and lymph-node metastasis were
examined, the level of MGP mRNA detected in the primary tumor was almost 5 times higher than in the lymph-node metastasis (Fig. 3a, samples TIP and TIL, respectively). The reduction of MGP expression found in the lymph-node metastasis was not due to dilution by normal lymphocytes since significant MGP expression was detected in the normal lymph node (Fig. 3a, sample 005). In addition, histological examination confirmed that, in the lymph-node metastasis (sample TIL of Fig. 3a), lymphatic tissue was completely replaced by undifferentiated tumor cells.

The MGP expression among the 3 types of urogenital malignancies examined, relative to the 600PEI cell line, is shown in Figure 4. The mean levels of MGP expression for these tumors were comparably high. The prostate carcinomas (PC) showed the highest mean MGP value (17.4 ± 5.5) as compared to renal-cell carcinomas (RCC; 15.3 ± 2.5) and testicular germ-cell tumors (TC; 14.5 ± 2.8). However, in prostate cancer cell lines (PCL) or primary breast carcinomas tested (BC; see also Fig. 1b), the MGP expression levels were undetectable or very low.

**DISCUSSION**

Matrix Gla protein was first isolated from bone and cartilage and was thought to be involved in bone formation (Fraser and Price, 1988). Evidence has emerged that MGP synthesis in osteoblasts is not dependent on mineralization but is associated with induction of the extracellular matrix formation (Barone et al., 1991). In non-osseous tissues the function of MGP has not been studied but it is likely that MGP may be associated with differentiation as well.

In the present study we have shown that primary tumors of the urogenital tract exhibited comparably high MGP mRNA levels. More specifically, 75% of renal-cell carcinomas and 55% of testicular germ-cell tumors over-expressed MGP as compared to normal matched tissues. In the prostate carcinomas, since no matched normal tissues were available for our analysis, we cannot state whether the high MGP expression observed reflected over-expression.

When correlated with clinical parameters, MGP expression appeared to be associated with more favorable prognostic features in renal-cell carcinomas. Low MGP mRNA levels were related with unfavorable clinical factors such as undifferentiated state, larger tumor size, and lymph-node metastasis. This result suggests that loss of MGP expression might correlate with a more malignant phenotype and perhaps with metastasis. An association between loss of MGP expression and tumor progression was further supported by our finding in prostate carcinomas. Prostate-cancer cell lines established from highly metastatic tumors did not express MGP. In addition, lymph-node metastasis had 5 times lower MGP mRNA levels than primary tumor. Southern analysis performed on the prostate carcinomas showed no amplification or other gross genetic rearrangement of the MGP gene to account for the differences in expression (data not shown). A metastatic phenotype has been previously correlated with down-regulation of the cell-adhesion molecules, cadherins (Takeichi, 1991), which might suggest a similar role for MGP in cell-cell or cell-matrix interactions.

Elevated MGP expression does not seem to be a common feature of carcinomas in general. For example, the primary breast carcinomas examined here exhibited only low MGP mRNA levels. In addition, prostate or breast-cancer cell lines (Chen et al., 1990), showed absent or very low MGP expression, suggesting that MGP is unlikely to represent a housekeeping gene, such as for example actin which shows ubiquitous expression among normal kidney tissues. This difference is not due to variations in tissue content (i.e., cortex vs. medulla), since these control tissues were uniformly removed from the cortical
regions, and confirmed histologically. It rather suggests variation of MGP expression in the general population, which could be due to physiological determinants, e.g., age, sex, exercise, diet and disease state, as has been reported for BGP, since both genes may respond similarly to hormonal stimuli (Hauschka et al., 1989; Cancela et al., 1990).

The implication of MGP as a vitamin-K-dependent protein of the extracellular matrix in malignancy is not surprising, since it has been suggested that vitamin-K-dependent proteins are involved in the metastatic process (Hauschka et al., 1986), and anti-metastatic effects of various anti-coagulants have been documented (Donati et al., 1986). In addition, perturbation of extracellular matrix components has been associated with invasion and metastasis (Liotta et al., 1991). Even though our analysis was based on a limited number of patients, the data suggest that MGP expression may be attenuated during the progression of some tumors.

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REFERENCES


