Characterization of Low Molecular Weight Protein Tyrosine Phosphatase Isoforms in Human Breast Cancer Epithelial Cell Lines

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Abstract. Background: Low molecular weight protein tyrosine phosphatase (LMW-PTP) is a polymorphic protein with two major isoforms whose role in tumorigenesis is currently controversial. Materials and Methods: We characterized LMW-PTP genotype, isoform expression and activity in six different human breast cancer epithelial cell lines (ZR75, MDA-MB-231, MDA-MB-231BO, MCF7, MDA-MB-231BO2, MDA-MB-435) and compared them with MCF10A, a normal breast epithelial cell line. Results: mRNA expression of the slow isoform was increased in almost all breast cancer cell lines and that of the fast isoform was reduced (p<0.05) in all breast cancer cell lines. Regarding enzymatic activity, only MCF7 had significantly lower (p<0.05) LMW-PTP activity compared to MCF10A. Conclusion: Since these are novel and previously unreported findings, we propose that the differential expression of LMW-PTP fast and slow isoforms suggests their opposite roles in the tumorigenic process, with the fast isoform being anti-oncogenic and the slow isoform being oncogenic.

Protein tyrosine phosphorylation is a key mechanism through which cells control vital functions such as cell growth, proliferation, motility and gene expression (2). This process is controlled by two groups of enzymes, protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). PTPs are a family of 107 enzymes that comprise of four classes (2). All enzymes share a common CX5R active site motif and an identical catalytic mechanism that is based on the participation of a crucial cysteine residue (25).

Overexpression of total LMW-PTP has been observed in many oncogene-transformed epithelial cell lines and is sufficient to transform non-transformed epithelial cells (16). The in vivo role of LMW-PTP in tumorigenesis has been analyzed by Chiarugi et al. (10), who evaluated the effect of overexpression of both total LMW-PTP and a dominant negative LMW-PTP on sarcoma development in nude mice. Total LMW-PTP was shown to be a positive regulator of both tumor onset and development in vivo. However, Malentacchi et al. analyzed a panel of human breast, colon and lung cancer surgical samples and their paired adjacent non-affected tissues, and observed that breast and colon cancer, but not lung cancer, exhibited increased mRNA levels for LMW-PTP (18).

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Given these discrepancies, and the fact that these studies only investigated total LMW-PTP, it is important to investigate the role of the two most abundant isoforms of LMW-PTP in different human cancer cell lines, and characterize their differential expression in these cells.

Materials and Methods

Cell culture. We characterized six different human breast cancer epithelial cell lines with respect to LMW-PTP genotype, isoform expression and activity. MCF10A, a spontaneously immortalized but non-transformed human breast epithelial cell line isolated from fibrocystic disease, is considered to be a normal breast epithelial cell line and was used as a comparator. All cell lines except MDA-MB-231 BO, MDA-MB-231 BO2 and ZR-75, were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). MDA-MB-231 BO, MDA-MB-231 BO2 and ZR-75 were kind gifts from the Division of Endocrinology and Metabolism (University of Texas Health Science Centre, San Antonio, Texas, USA), INSERM Research Unit 403 (Faculté de Medecine Laennec, Lyon, France), and the University of Virginia, USA, respectively. MCF10A was cultured in Clonetics mammary epithelial cell growth medium (MEGM) supplemented with BulletKit (CC-3150) (Lonza, Basel, Switzerland) and 100 ng/ml cholera toxin. MDA-MB-231 is derived from a breast adenocarcinoma and was cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. MDA-MB-435 (Hs00246642_m1). Results were normalized to real-time RT-PCR of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the Human GAPDH Endogenous Control (Applied Biosystems).

Genotyping. Cells were cultured in 6-well plates and incubated in serum-free medium for 48 h prior to experiments. DNA was isolated from cells at 90% confluence using the Easy Spin® Nucleic Acid Extraction kit (Citomedia, Lisbon, Portugal) and quantified with Nanodrop (ThermoScientific, Waltman, MA, USA). DNA was digested with two different restriction enzymes, Hin6I and MspA1.

LMW-PTP mRNA expression. Cells were cultured in 6-well plates and incubated in serum-free medium for 48 h prior to experiments. Total cellular RNA was isolated from cells at 90% confluence with the RNeasy mini kit (Qiagen, Foster City, CA, USA). RNA was quantified by absorbance at 260 nm, and purity determined by absorbance at 280 and 310 nm with NanoDrop (ThermoScientific). RNA (1 μg) was converted into cDNA using the QuantiTec® Reverse Transcriptase kit (Qiagen, Foster City, CA, USA). An aliquot (0.2 μl) of the cDNA was amplified in an ABI Prism 7000 real-time RT-PCR unit using the following TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA): ACP1 total (Hs00962877_m1); ACP1 fast isoform (Hs00964348_g1); ACP1 slow isoform (Hs00246642_m1). Results were normalized to real-time RT-PCR of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the Human GAPDH Endogenous Control (Applied Biosystems).

Enzymatic activity. Cells were cultured in 96-well plates and incubated in serum-free medium for 48 h prior to experiments. Ex vivo activity of LMW-PTP was measured in cells at 60%-70% confluence according to the method of Balcerzyk et al. (4). Briefly, lysis buffer, containing 10 mM p-nitrophenyl phosphate and 0.1% Triton X-100 in 0.1 M sodium acetate, 10 mM EDTA (pH 5.5) was added to the cells for 2 h. Samples were alkalized by adding 1 M NaOH and the absorbance of p-nitrophenol was then measured at 405 nm. Results were normalized to total protein content, determined by Precision Red™ Advanced Protein Assay Reagent (Cytoskeleton, Denver, CO, USA). This method measures total LMW-PTP enzymatic activity. There is currently no method capable of differentiating between the activity of the different isoforms.

Statistical analysis. All data are expressed as the mean±standard deviation. Significance was established by the Student’s t-test or ANOVA and post-hoc Sidak test, where appropriate. Differences were considered significant at p<0.05.

Results

ACP1 genotype of human breast epithelial cell lines. ACP1 has three different alleles, A, B, and C, thus giving rise to six possible genotypes. ACP1 genotype of each of the studied cell lines is presented in Table I, showing that only three out of the six possible ACP1 genotypes are present, AA, AB and BB.

LMW-PTP mRNA expression in human breast epithelial cell lines. Figure 1 shows the relative expression of total LMW-PTP and its fast and slow isoforms in the studied tumor cell
lines compared to MCF10A. There was an increased expression of the slow isoform in almost all breast cancer cell lines and a reduced expression of the fast isoform in all breast cancer cell lines. Expression of total LMW-PTP was only reduced in the ZR-75 cell line.

**LMW-PTP activity in human breast epithelial cell lines.** Enzymatic activity of LMW-PTP depends not only on the ACP1 genotype but also on the presence of inhibitors and activators (17). The results show that only MCF7 had a lower LMW-PTP activity when compared to MCF10A (Figure 2).
Discussion

There are various studies associating genetic polymorphisms of *ACP1* with different pathologies, *e.g.* developmental disturbances and hemolytic favism (6); systemic lupus erythematosus (14); obesity-related hypertension (11) and cancer (1, 23). *ACP1* genotyping in patients with different types of cancer has shown a positive association of cancer with genotypes carrying the B allele (1) and a decrease of genotypes carrying the C allele (23). In our cell lines, only three out of the six possible *ACP1* genotypes were present, AA, AB and BB. These are the most frequent genotypes and the ones that seem to be positively-correlated with cancer (1, 23).

Malentacchi et al. (18) studied the expression profile of LMW-PTP in a large panel of human tumors and their paired adjacent normal tissue and concluded that overexpression of LMW-PTP mRNA leads to over-production of LMW-PTP protein. Their results suggest that LMW-PTP overexpression occurs in breast cancer and is correlated with the aggressiveness of the tumor. The fact that the expression of total LMW-PTP was only lower in the ZR-75 cell line when compared to MCF10A, with no changes in the other tumor cell lines, may reflect the presence of all other isoforms, *fast* and *slow* are not the only isoforms of LMW-PTP (19, 21). Therefore, the fact that total LMW-PTP expression is not different from MCF10A, although there is an alteration in both the fast and slow isoforms expression, can be due to the contribution of the other isoforms.

However, there was an increase of the slow isoform expression in almost all cancer cell lines and a reduction of expression of the fast isoform in all tumor cell lines, compared to MCF10A. This leads us to suggest that this differential expression may be associated with the tumorigenic potential of the cells. A possible mechanism that could explain the pronocogenic potential of the slow isoform is the dephosphorylation potential of membrane receptor proteins this isoform has (8). Given previous reports suggesting that dephosphorylated EPHA2 favors transformation of normal epithelial cells and influences tumor growth (22), it may be hypothesized that the slow isoform can increase the tumorigenic potential through de-phosphorylation of EPHA2. Regarding the decrease in mRNA expression of the fast isoform, this can reduce the phosphorylation status of Ras homolog gene family, member A (RhoA), through p190RhoGAP, rendering it more inactive (GDP-bound conformation). This modification alters cell adhesion and, consequently, the migratory ability of the cells. Therefore, LMW-PTP isoforms may be involved in the tumorigenic process by interfering with cell growth, adhesion and migration, which are hallmarks of cancer. Taken together, these results suggest a potential role of LMW-PTP fast and slow isoforms as prognostic markers and possible therapeutic targets in cancer.

Regarding LMW-PTP enzymatic activity, our results showed that only the MCF7 cell line had a lower LMW-PTP activity when compared to MCF10A. This is not consistent with the mRNA expression, which showed that only the ZR-75 cell line has a lower total LMW-PTP expression. Therefore, it would be expected that this cell line would have a lower total enzymatic activity. We have no straightforward explanation for these results since they have not been reported previously. We can hypothesize that in the ZR-75 cell line, the LMW-PTP protein can be directly activated or stabilized, its mRNA stabilized or the mRNA translation rate increased. Moreover, and regarding post-translational modifications, phosphorylation of Tyr131 and Tyr132 can induce an increase of LMW-PTP activity (9). Given LMW-PTP is finely-regulated in tumor cells, several of these mechanisms, either alone or synergistically, may be sufficient to explain the observed differences.

Regarding MCF7 results, MCF7 is a non-metastatic breast tumor cell line that seems to have different characteristics compared to the other studied cell lines. These cells grow more slowly, more clustered and have epithelial characteristics, since they do not express vimentin nor N-cadherin, two important mesenchymal markers (5). These intrinsic characteristics of the cells can interfere with LMW-PTP activity measurement since substrate availability may be low due to limited access to all cells: although the number of cells is the same, the fact that they grow closely in clusters may hinder the efficacy of cellular lysis and consequently limit substrate access. The fact that this cell line exhibits a more epithelial-like phenotype, similar to MCF10A, unlike ZR-75, means that other factors, such as purines, folic acid and pirimidines (17), may be involved in the regulation of LMW-PTP activity. Moreover, our results suggest novel functions of LMW-PTP isoforms in tumorigenesis, showing that the expression of these isoforms is different among human breast cancer epithelial cells, with an overexpression of the slow isoform in almost all of the studied tumor cell lines and reduced expression of the fast isoform in all tumor cell lines. This leads us to propose that these two isoforms have opposing roles in the tumorigenic process, with the slow isoform being oncogenic and the fast isoform anti-oncogenic, which can explain the previous contradictory findings regarding the role of LMW-PTP in cancer. Furthermore, we propose that LMW-PTP isoforms may be considered prognostic markers of the tumorigenic process, and possible therapeutic targets. Given these are novel and previously unreported findings, we are currently conducting studies to explore the possible differential role of LMW-PTP isoforms in the tumorigenic process.

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