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Specific Subcellular Localization of Phosphoinositide-Specific Phospholipase C enzymes in Different Human Osteosarcoma Cell Lines

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Abstract

The role of signal transduction in cancer progression is well established and actively studied, including in osteosarcoma. The signal transduction pathways involved in the regulation of calcium metabolism are being intensively studied, with particular regard to phosphoinositide-specific phospholipase C (PLC) signaling. This family of enzymes helps to modulate calcium metabolism and is interconnected with additional signaling molecules belonging to different pathways. The expression and subcellular localization of PLCs have been shown to differ in normal cells compared to their neoplastic counterpart in different types of cancer. We now describe the localization of the PLC enzyme family in 4 human osteosarcoma cells different in origin and malignancy (MG63, U2OS, HOS and 143B cell lines). We identified cell line-specific differences and discussed possible meaning and implications.

Keywords: Phospholipase C, Phosphoinositide, Osteosarcoma, 143B, HOS, U2OS, MG63

Introduction

The primary bone tumors account for 0.2% of cancers and 0.3% mortality in cancer deaths [1]. Among these, the most frequent is osteosarcoma (OS), which accounts for 17% of all bone tumors [1] and affects 5 million persons per year [2] with different gender incidences and bimodal age distribution [3,4]. OS is the most diffused primary bone tumor in the youngest population [2], accounting for 5% of cancers and 9% of cancer-related deaths in childhood [4]. OS comprises tumors slightly differing for clinical, radiological, and histological features [3,5,6]. The wide number of abnormalities observed in OS made it difficult to define the diagnosis, and the prognosis, and to schedule the appropriate therapy [7]. Although many efforts were spent, the etiology, pathogenesis, and natural history of OS are not completely clarified.

A recent effort was represented by investigations addressed to define the number and nature of signal transduction pathways networking in the pathogenesis of OS, including the role of Phosphoinositide-specific Phospholipase C (PLC) family of enzymes, which contribute to the regulation of intracellular calcium concentration [8]. Moreover, PLCs regulate the metabolism of Phosphatydil inositol (4,5) bisphosphate (PIP2), a minority acidic membrane phospholipid critical for many cellular activities, and by cleaving this important molecule, can generate two important second messengers, inositol trisphosphate (IP3) and diacylglycerol (DAG), both crucial molecules in signal transduction [9]. Calcium metabolism was demonstrated to be critical for cells viability in human OS cell lines [10], and previous literature data reported anomalies in the expression of selected *PLC* genes or PLC enzymes in a number of OS cell lines [11-17].

In the present paper, we identified the presence of transcripts from *PLC* genes, the presence of PLC enzymes and intracellular

location in 4 different cultured OS human cell lines, namely MG63, HOS, U2OS, and 143B.

Materials and Methods

Cell culture

Osteosarcoma cell lines HOS, 143B, MG63, U2OS were obtained from Deutsche Sammlung von Mikroorhanismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). Cells were cultured in T25 Flask for molecular biology and on a glass coverslip in Multiwell 24 plate for the fluorescence immunocytochemistry experiments. Cells were cultured at 37°C with 5% CO_2 in DMEM 1 mg/l Glucose (Sigma-Aldrich, Missouri, USA), supplemented with 10% fetal bovine serum (GIBCO, Thermofisher Scientific Inc, Massachussets, USA), penicillin (100 µg/ml) and streptomycin (100 U/ml) (Sigma-Aldrich). The cell seeding density was 4,000 cells/cm^2. For molecular biology studies, cells were splitted 1:10 every three days. For molecular biology the culture stopped on day 2. For fluorescence immunocytochemistry the timepoints were fixed at day 0 and day 2.

RT-PCR

Cells were collected and processed with TRIZOL reagent (Invitrogen, Thermofisher Scientific Inc), then the total nucleic acids were extracted with isopropanol-chloroform methodology (according to TRIZOL manufacturer instructions). Then the RNA was quantified by using the Nanodrop Spectrophotometer (Thermofisher Scientific Inc), using UV absorption ratio 260: 280. After that, 1 µg of total RNA was reverse transcribed with the High-Capacity Transcription Kit by Thermofisher Scientific. GoTaq colorless master mix by Promega (Promega Corp, Wisconsin, USA) was used to amplify the cDNA. The PCR was set according to manufacturer instructions: the reaction started with an initial denaturation step at 94°C for 2 min, followed by 30 cycles which consist in (1) denaturation (30 s) at 95°C, (2) annealing (30 s) at the appropriate temperature for each primer pair and (3) extension (1 min) at 72°C. The PCR products were analyzed by electrophoresis on agarose gel 1.5% in TBE stained with Gel Red (Biotium). These primer pairs were used (DBA):

PLCB1 (OMIM *607120) forward (f) 5′-AGCTCTCAGAACAAG CCTCCAACA-3′, reverse (r) 5′-ATCATCGTCGTCGTCACTTTC CGT-3′; PLCB2 (O MIM*604114) (f) 5′-AAGGTGAAGGCCTATCT GAGCCAA-3′, (r) 5′-CTTGGCAAACTTCCCAAAGCGAGT-3′; PLCB3 (OMIM* 600230) (f) 5′-TATCTTCTTGGACCTGCTGAC CGT-3′, (r) 5′-TGTGCCCTCATCTGTAGTTGGCTT-3′; PLCB4 (OMIM *600810) (f) 5′-GCACAGCACACAAAGGAATGGTCA-3′, (r) 5′-CG CATTTCCTTGCTTTCCCTGTCA-3′; PLCG1 (OMIM *172420) (f) 5′-TCTACCTGGAGGACCCTGTGAA-3′, (r) 5′-CCAGAAAGAGAG CGTGTAGTCG-3′; PLCG2 (OMIM *600220) (f) 5′-AGTACATG CAGATGAATCACGC-3′, (r) 5′-ACCTGAATCCTGATTTGAC TGC-3′; PLCD1 (OMIM *602142) (f) 5′-CTGAGCGTGTGGTTCCAGC-3′, (r) 5′-CAGGCCCTCGGACTGGT-3′; PLCD3 (OMIM *608795) (f) 5′-CCAGAACCACTCTCAGCATCCA-3′, (r) 5′-GCCATTGTTGAG CACGTAGTCAG-3′; PLCD4 (OMIM *605939) (f) 5′-AGACAC GTCCCAGTCTGGAACC-3′r 5′-CTGCTTCCTCTTCCTCATATTC-3′; PLCE (OMIM *608414) (f) 5′-GGGGCCACGGTCATCCAC-3′, (r) 5′-GGGCCTTCATACCGTCCATCCTC-3′; PLCH1 (OMIM *612835) (f) 5′-CTTTGGTTCGGTTCCTTGTGTGG-3′, (r) 5′-GGATGCTTCT GTCAGTCCTTCC-3′; PLCH2 (OMIM *612836) (f) 5′-GAAACTGG CCTCCAAACACTGCCCGCCG-3′, (r) 5′-GTCTTGTTGGAGATGCAC GTGCCCCTTGC-3′; GAPDH (OMIM * 138400) (f) 5′-CGAGATC CCTCCAAAATCAA-3′; (r) 5′-GTCTTCTGGGTGGCAGTGAT-3′. The specificity of the primers was insured through research into the NCBI database for possible homology to cDNAs of unrelated proteins.

Table 1. PCR primers' list.

GENES	OMIM	Forward Sequence	Reverse Sequence
PLCB1	*607120	5′-AGCTCTCAGAACAAGCCTCCAACA-3′	5′-ATCATCGTCGTCGTCACTTTCCGT-3′
PLCB2	*604114	5′-AAGGTGAAGGCCTATCTGAGCCAA-3′	5′-TTGGCAAACTTCCCAAAGCGAGT-3′
PLCB3	*600230	5′-TATCTTCTTGGACCTGCTGACCGT-3′	5′-TGTGCCCTCATCTGTAGTTGGCTT-3′
PLCB4	*600810	5′-GCACAGCACACAAAGGAATGGTCA-3′	5′-CGCATTTCCTTGCTTTCCCTGTCA-3′
PLCG1	*172420	5′-TCTACCTGGAGGACCCTGTGAA-3′	5′-CCAGAAAGAGAGCGTGTAGTCG-3′
PLCG2	*600220	5′-AGTACATGCAGATGAATCACGC-3′	5′-ACCTGAATCCTGATTTGACTGC-3′
PLCD1	*602142	5′-CTGAGCGTGTGGTTCCAGC-3′	5′-CAGGCCCTCGGACTGGT-3′
PLCD3	*608795	5′-CCAGAACCACTCTCAGCATCCA-3′	5′-GCCATTGTTGAGCACGTAGTCAG-3′
PLCD4	*605939	5′-AGACACGTCCCAGTCTGGAACC- 3′	5′-CTGCTTCCTCTTCCTCATATTC- 3′
PLCE	*608414	5′-GGGGCCACGGTCATCCAC-3′	5′-GGGCCTTCATACCGTCCATCCTC-3′
PLCH1	*612835	5′-CTTTGGTTCGGTTCCTTGTGTGG-3′	5′-GGATGCTTCTGTCAGTCCTTCC-3′
PLCH2	*612836	5′-GAAACTGGCCTCCAAACACTGCCCGCCG-3′	5′-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3′

The Gel image acquisition occurred by the Chemidoc Imaging System by Biorad. To ensure PCR non-contamination a negative control was performed. Optical densities of the samples were normalized on RNA transcript of glyceraldehyde-3-phosphate dehydrogenase human gene (GAPDH).

Fluorescence immunocytochemistry

Cells grown on glass coverslips were fixed for 10 minutes with paraformaldehyde 4% (PFA) and then washed three times with phosphate buffer saline (PBS). Cells were incubated overnight (ON) at 4°C with anti PLCs primary antibodies diluted at 1:200 in PBS. Mouse primary antibodies (PLC β, PLC γ and PLC δ sub-families) from Santa Cruz (Santa Cruz Biotechnology, Inc, Texas, USA) were detected with appropriate fluorescent dye conjugated anti-mouse secondary antibodies from Bethyl Laboratories (Bethyl Laboratories Inc, Fortis Life Sciences, Texas, USA). Rabbit primary antibodies, from Invitrogen and SIGMA (PLC ε and PLC η sub-family), were detected with appropriate fluorescent dye conjugated anti-rabbit secondary antibodies from Thermofisher Scientific. Both secondary antibodies were diluted 1:200 in PBS and incubated for 1 hour at 4°C in the dark. Next, cells were washed twice with PBS for 5 minutes and then counterstained with 4',6-diamidino-2-phenylindole (DAPI) fluorescent staining for 20 minutes at RT in the dark. After three more washes in PBS the coverslips were mounted and visualized with a Nikon fluorescence microscope equipped with NIS Elements software (Nikon, Japan).

Results

Molecular biology

Transcripts from all *PLC* genes were identified in the 4 OS cell lines (**Table 2, Figure 1**). The transcript of *PLCH2* was invariably detected as a double band in all the analyzed cell lines (**Table 2, Figure 1**). In MG63, the transcripts of *PLCG1*, and *PLCD3* were also detected as a double band. In U2OS, transcripts from *PLCG1* and *PLCG2* were also detected as double band. In HOS, also the transcript from *PLCG2* was detected as a double band (**Table 2, Figure 1**).

Fluorescence microscopy

In MG63 cells, PLC β3, PLC β4, PLC δ3, and PLC δ4 were not detected, and PLC g isoforms were very slightly detected. PLC β1 was detected in the nucleus and as a wide perinuclear halo and in cytoplasm vacuoles. PLC δ1 was detected in the nucleus and in cytoplasm vacuoles. PLC η was detected in the cytoplasm and slightly in the nucleus. PLC η isoforms were exclusively detected in the cytoplasm, cytoplasmic protrusions, with special regard to PLC η2 enzyme, also identified in cytoplasm vesicles and in pseudopod-like structures (**Table 3, Figures 2 and 3**).

In U2OS cells, PLC enzymes were mainly detected in the cytoplasm. PLC β1 was slightly detected in the nucleus and cytoplasm, and better visible in cell protrusions (**Table 3,**

Table 2. PCR results.

OS cell line	PLC β1	PLC β2	PLC β3	PLC β4	PLC γ1	PLC γ2	PLC δ1	PLC δ3	PLC δ4	PLC ε	PLC η1	PLC η2
MG-63	+	+	+	+	+, db	+	+	+, db	+	+	+	+, db
U2OS	+	+	+	+	+, db	+, db	+	+	+	+	+	+, db
HOS	+	+	+	+	+	+, db	+	+	+	+	+	+, db
143B	+	+	+	+	+	+	+	+	+	+	+	+, db

+: presence of transcript; db: double electrophoretic band.

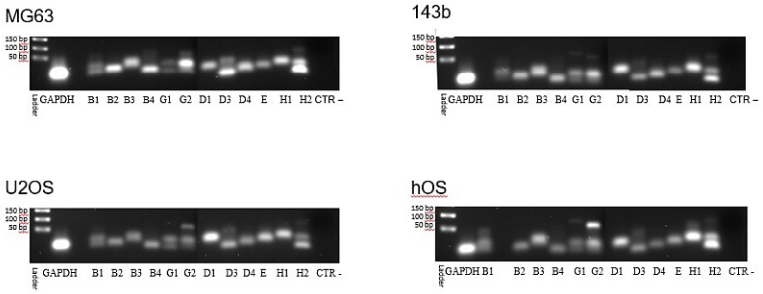

Figure 1. Gel electrophoresis of PCR results.

Table 3. Results of fluorescence immunocytochemistry experiments for the intra-cellular localization of PLC enzymes.

OS cell line	PLC β1	PLC β2	PLC β3	PLC β4	PLC γ1	PLC γ2	PLC δ1	PLC δ3	PLC δ4	PLC ε	PLC η1	PLC η2
MG-63	N Cpnw V	C	-	-	N -/+ C -/+	N -/+ C -/+	N C V	-	-	N-/+ C	C Pr	C vv Pr pspod
U2OS	N -/+ C -/+ Pr	C vv Pr Pspod	INdep Cpns -/+ vv	Cpnw -/+	C-/+ Pr	C -/+ Pr	C -/+ Pr	C-/+ vv Pr Mru	Pr Mp	C -/+	N-/+ Pr Mp	N-/+ C-/+
HOS	C -/+ pM	C dep	C Pr pspod	N? C Pr	C V pspod	C Pr pspod	N dep Cpnw lindep	INdep C Pr pspod	N -/+ C -/+	C -/+	INdep C -/+	C vv punct
143B	C V	nM C pspod	INdep C V pspod	C V Pr	C pspod	C Pr pspod	C	C vv V pM	C V	C Pr	C Pr	N C V Pr

N: intranuclear; INdep: Uneven distributed in intranuclear deposits; C: cytoplasmic; V: vacuoles; vv: vesicles; pn: perinuclear cytoplasmic distribution; Cpns: subtle perinuclear cytoplasmic halo; Cpnw: wide perinuclear cytoplasmic halo; dep: deposits; lindep: linear shaped deposits; Pr: cell protrusions; punct: punctuate distribution (vesicles); pM: plasma membrane; nM: nuclear membrane; comp: compartmentalized position in the cell; fil: filamentous distribution; pspod: pseudopodia-like structures; Mru: membrane ruffles. -/+: weak fluorescence signal.

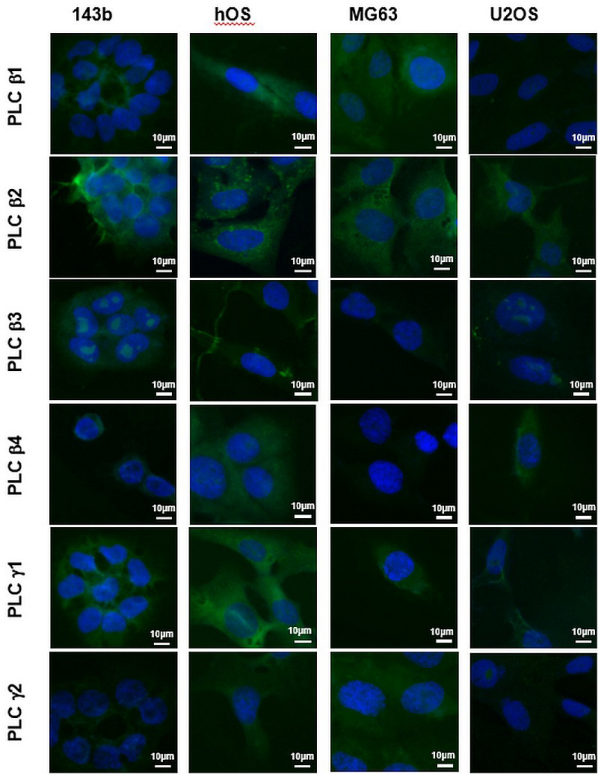

Figure 2. Fluorescence immunocytochemistry. Detection of PLC enzymes belonging to β and γ sub-families presence, counterstained with DAPI (Blue nuclei).

Figures 2 and 3). PLC β2 was detected in cytoplasm vesicles, in cell protrusions and pseudopod-like structures. PLC β3 was detected in the nucleus as intranuclear deposits, and in the cytoplasm as a subtle perinuclear cytoplasmic halo and in cytoplasmic vesicles. PLC β4 was slightly detected as a wide perinuclear cytoplasmic halo. PLC γ isoforms and PLC δ1 were slightly detected in the cytoplasm, and were detected in cell protrusions (**Table 3, Figure 2 and 3**). Similarly, PLC δ3 was slightly detected as a cytoplasm halo and in cytoplasmic vesicles, in cell protrusions and in membrane ruffles. PLC δ4 was detected in cell protrusions and at the plasma membrane. PLC ε was slightly detected in the cytoplasm. PLC η1 was slightly detected in the nucleus, and was detected in cell protrusions and at the plasma membrane. PLC η2 was slightly detected both in the nucleus and in the cytoplasm (**Table 3, Figures 2 and 3**).

In HOS cells, PLC β1 was slightly detected in the cytoplasm and at the plasma membrane (**Table 3, Figure 2 and 3**). PLC β3 was detected in cytoplasm deposits, and both PLC β3 and PLC γ2 were detected in cytoplasm, in cell protrusions and pseudopod-like structures. PLC β4 was inconstantly detected in the nuclei and was detected in cytoplasm and cell protrusions. PLC γ1 was detected in cytoplasm and in cytoplasmic vacuoles, as well as in cell protrusions (**Table 3, Figures 2 and 3).** PLC γ1 was detected in nuclear deposits, and as a wide perinuclear cytoplasmic halo and in linear shaped deposits. PLC δ3 was detected in the nucleus as intranuclear deposits, and in the cytoplasm, cell protrusions and pseudopod-like structures (**Table 3, Figures 2 and 3**). PLC δ4 was slightly detected both in the nucleus and in the cytoplasm, and PLC ε exclusively in the cytoplasm. PLC η1 was detected in the nucleus as intranuclear deposits and slightly also in the cytoplasm. PLC η2 was detected in the cytoplasm with a punctuate distribution (**Table 3, Figures 2 and 3**).

In 143B cells, PLC β1 was detected in cytoplasm vacuoles (**Table 3, Figures 2 and 3**). PLC β3 was detected at the nuclear membrane, in the cytoplasm, and in pseudopod-like structures. PLC β3 was detected in intranuclear deposits, in cytoplasm vacuoles and in pseudopod-like structures. PLC β4 was detected in cytoplasm vacuoles and in cell protrusions (**Table 3, Figures 2 and 3**). PLC γ1 and PLC γ2 were detected in the cytoplasm and in pseudopod-like structures, whilst PLC γ2 was detected also in cell protrusions (**Table 3, Figures 2 and 3**). PLC δ1 was detected in the cytoplasm. PLC δ3 was detected in the cytoplasm in vacuoles and vesicles, and at the plasma membrane. PLC δ4 was detected in cytoplasm vacuoles. PLC ε and PLC η1 were detected in the cytoplasm and in cell protrusions. PLC η2 was detected in the nucleus, in the cytoplasm with a vacuolar distribution, and in cell protrusions (**Table 3, Figures 2 and 3**).

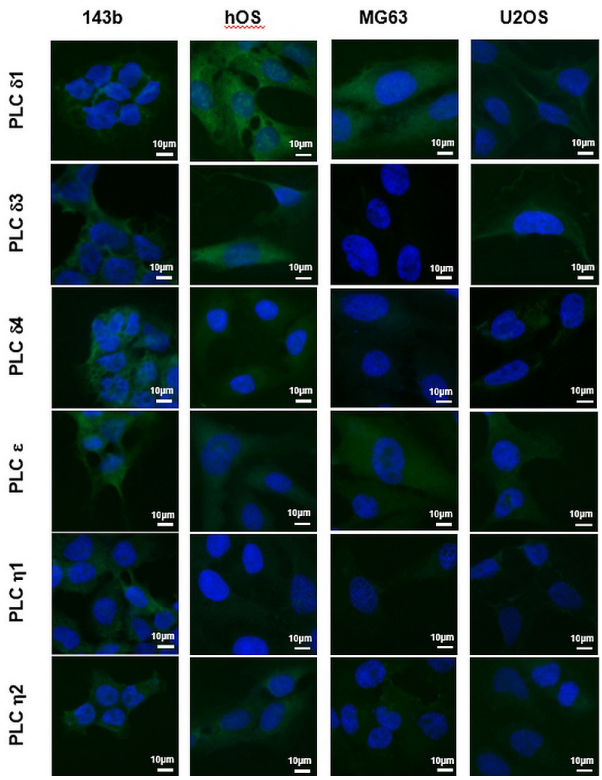

Figure 3. Fluorescence immunocytochemistry. Detection of PLC enzymes belonging δ, ε, and η sub-families presence, counterstained with DAPI (Blue nuclei).

Discussion

The role of signal transduction is considered crucial in the progression of osteosarcoma, with special regard to the pathways involved in the regulation of calcium metabolism, such as PLC signaling. In fact, the complex regulation of calcium is critical to many physiological events, as abnormal increase can affect protein activities, apoptosis, secretion mechanisms, and contractile activity [18]. Calcium metabolism was demonstrated to be critical for the viability of human OS cell lines [5,6].

PLC enzymes were actively studied in human osteosarcoma as they are involved in calcium signaling. PLC enzymes act upon Phosphatydil inositol (4,5) bisphosphate (PIP2), cleaving the polar head and producing two further signaling molecules, inositol trisphosphate (IP3) and diacylglycerol (DAG) [19]. IP3 is water-soluble, and after diffusion in the cytoplasm, IP3-gated calcium channels induce calcium release from the endoplasmic reticulum (ER) membrane [20]. DAG binds to the cell membrane and is cleaved into arachidonic acid to synthesize eicosanoids. DAG also activates the serine/threonine calcium-dependent protein kinase C (PKC) family of enzymes [19]. Besides the role upon calcium signaling, interest arose in PLC enzymes for their ability to reduce the levels of PIP2.

PIP2 represents a crucial molecule in many physiological aspects. In osteosarcoma the activation of proteins belonging to the Ezrin, radixin, moesin homology (ERM) family requires local accumulation of PIP2. Ezrin, a protein belonging to the ERM family, is considered a crucial molecule in osteosarcoma. Reduction of ezrin significantly reduced the metastatic spread in animal models [21-23]. Ezrin is mainly involved in actin assembly [24,25], depending upon membrane levels of PIP2 [26]. While inactive Ezrin is located in the cytoplasm, once activated ezrin moves, and tethers actin to the cortical membrane. That induces cell morphology alterations following cytoskeleton reorganization [27-32]. Besides phosphorylation, activation of ezrin also occurs after interaction with PIP2 [33-36]. The competition of ezrin and PLC for PIP2 suggested the hypothesis that PI signaling might allow highlight the role of ezrin in metastatic spread and pave the way to novel therapy perspectives.

The reduction of PIP2 levels, which occurs after cleavage by PLC, induces ezrin to dissociate from the cell membrane [37].

In the present paper, we analyzed the expression and intracellular localization of PLC enzymes in four human OS cell lines, bearing different grades of malignancy. U2OS cell line was derived from a moderately differentiated osteosarcoma obtained from the tibia of a 15-year-old Caucasian girl. MG-63 cells are a type of osteoblast cell line with fibroblast morphology isolated from the bone of a 14-year-old White male patient with osteosarcoma and are widely used as osteoblast experimental model. HOS is a fibroblast and epithelial-like cell line that was isolated from the bone of a White, 13-year-old, female patient with osteosarcoma. The 143B cell line is a derivative of the HOS cell line, which was established from a tumor of a 13-year-old girl. HOS and 143B cell lines were demonstrated to be highly metastatic to the lungs of the animals after subcutaneous and intramuscular xenotransplantations [38].

In our present experiments, MG-63 cells lack the presence of selected PLC enzymes, including PLC δ3. Recent literature data report that PLC δ3 was not detected in human osteoblasts [39,40]. PLC δ3 in involved in physiological processes, such as the downregulation of RhoA/Rho signaling during neurite extension [41], and in cardiomyocyte survival [42]. PLC δ3 was indicated as an oncogene in nasopharyngeal carcinoma [43] and breast cancer [44]. Moreover, in thyroid cancer PLC δ3 was demonstrated to inhibit apoptosis and to promote the proliferation, migration and invasion by networking the Hippo pathway [45]. The expression of *PLCH2* and, inconstantly, of *PLCG2* genes was detected as a double electrophoretic band, comprised of two bands of slightly different molecular weight, suggesting that two splicing isoforms were transcribed. The alternative presence of splicing isoforms, with different localization, was described for different PLC isoforms [19].

In MG-63 most PLC enzymes were detected both in the nucleus and in the cytoplasm, with the notable exception of PLC η enzymes, exclusively detected in the cytoplasm and in cell protrusions. Our present observations might corroborate the use of MG-63 cells as osteoblast experimental model also for the investigations about PLC signaling.

The number of PLC enzymes detected within the nucleus decreases in U2Os cells, and even more in HOS and in 143B cells. That might suggest that the presence of PLC enzymes in the nucleus is reduced and in the cytoplasm is enhanced depending on the malignancy grade. Moreover, PLC enzymes were also detected in cell protrusions, pseudopod like structures, and membrane ruffles. Plasma membrane ruffling and pseudopods represent a feature of migrating cells and might be related to calcium signaling. The presence of selected PLC enzymes in membrane ruffles in OS cell lines might suggest the involvement in cell migration and, as far one can see, in metastasization.

Selected PLC enzymes were detected in pseudopod-like structures, in cell protrusions, and/or in membrane ruffles (**Table 3, Figures 2 and 3**). This observation accords to previous literature data describing PLC enzymes in pseudopod-like structures [39,40,46,47], probably suggesting that PLC enzymes might be involved in in cell cytoskeleton reorganization. The presence and distribution of pseudopod-like structures we identified in the present experiments increased depending on the malignancy potential of the analyzed OS cells lines. The growing presence of PLC enzymes

in pseudopod-like structures, and in cell protrusions might suggest a role for the PLC signaling in the formation of cell structures potentially involved in cell motility and, as far as one can see, in cell migration. That might be of great interest, with special regard to 143B cells, known to be able to form metastases.

Selected PLC enzymes were detected in intracellular vesicles in all the analyzed OS cell lines, accordingly to previous literature data that identified the presence of PLCs both in intracellular vesicles in osteoblasts [39,40] and in extracellular vesicles in different OS cell lines [17].

Interestingly, in 143B cells, selected PLC enzymes were detected in intracytoplasmic vacuoles. In 143B cells, large intracytoplasmic vacuoles were described following rasfonin treatment inducing autophagy and activation of caspase-dependent apoptosis concurring with suppression of cell proliferation and migration [48]. Intracytoplasmic vacuoles in 143B cells were also described after treatment with nembolide, a limonoid known to have cytotoxic activity [49]. Thus, PLC enzymes were not represented in the nucleus of 143B malignant cells, more than in other OS cell lines. This observation might suggest that the intranuclear presence of selected PLCs might contribute to regulate the cell cycle, dysregulated in malignant transformation. Further studies are required in order to investigate this promising observation.

Finally, many evidences suggest the alternate presence of selected PLC isoforms in the cell nucleus and cytoplasm. Little is known about regulation, function and possible subnuclear localization of different PLC isoforms, such as described for PLC β1, PLC γ1, and PLC δ localization [19]. More recently, the compartmentalization of inositol lipid metabolism within the cell nucleus was demonstrated [50-52]. The complex regulation of cell compartments and the role of subcellular localization of PLC enzymes is not fully clear. Previous reports suggest that PLC enzymes localize differently in the normal tissue with respect to pathological counterparts. Thus, intracellular PLC's localization might affect regulation, representing a promising perspective to investigate the possible role in physiological activities and/or in malignant transformation of cells.

Thus, the localization of PLC enzymes was described to affect the function, and further investigations are required in order to highlight the reduction of intranuclear presence of PLC and possible relationship with different malignancy features of OS cell lines.

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