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L-type voltage-dependent Ca^{2+} channels expression involved in pre-neoplastic transformation of breast cancer

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Abstract

Background: Intracellular Ca^{2+} levels can modulate several cellular functions, including proliferation and other processes found altered in neoplastic cells. Helping to maintain Ca^{2+} homeostasis, L-type voltage-dependent Ca^{2+} channels had its expression identified in neoplasias, including breast cancer. Invasive breast carcinoma of no special type, the most common classification of breast cancer, has ductal hyperplasia and ductal carcinoma *in situ* as its possible non-obligate precursors. This channel's role in breast cancer development from these precursors has not been investigated. Evaluate protein expression and subcellular localization of $Ca_V1.1$, $Ca_V1.2$, and $Ca_V1.3$ in mammary epithelium without alteration and neoplastic and non-neoplastic ductal proliferative lesions through immunohistochemistry was the aim of this investigation.

Methods: In the present study, $Ca_V1.1$, $Ca_V1.2$, and $Ca_V1.3$ protein expression was evaluated by immunohistochemistry in breast without alteration and in proliferative non-neoplastic and neoplastic ductal epithelial lesions of the human breast.

Results: It was observed that $Ca_V1.3$ presented a reduction in nuclear expression at neoplastic lesions, in addition to an increase in cytoplasmic $Ca_V1.1$ expression. The analyses of membrane immunostaining showed that $Ca_V1.2$ and $Ca_V1.3$ had an increase of expression as the lesions progressed in the stages leading to invasive carcinomas.

Conclusions: Changes in protein expression and subcellular localization of these channels during the progression stages indicate that they may be involved in neoplastic transformation.

Keywords: Breast cancer, Ductal hyperplasia, Carcinoma *in situ*, L-type voltage-dependent Ca^{2+} channel, LTCC, Neoplastic transformation

Background

Considered an important second messenger, Ca^{2+} ions can assist in regulation of fertilization, secretion, contraction, transcription and phosphorylation through its role in different intracellular signalling pathways (Berridge et al., 2000). Ca^{2+} homeostasis must be maintained properly to avoid the development of cells with malignant features (Déliot and Constantin, 2015). Therefore, Ca^{2+} can be released from intracellular organelles, like the endoplasmic reticulum, or enter into the cell from the extracellular space through ion channels, such as voltage-dependent Ca^{2+} channels (Berridge et al., 2003).

L-type Ca^{2+} channels (LTCC), a voltage-dependent Ca^{2+} channel sub-family, have long-lasting Ca^{2+} currents and requires a strong membrane depolarization for activation (Catterall et al., 2005). Their molecular structure consists of an α1 subunit, responsible for transmembrane pore assembling and for the main biophysical and

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functional properties of the channel. This subunit can be encoded by four different genes, CACNA1S, CACNA1C, CACNA1D, CACNA1F, that originates the four isoforms of these channels, $Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$ e $Ca_V1.4$, respectively (Zamponi et al., 2015). In addition, α1 can be associated with other subunits, β, α2δ, and γ, that control channel's expression and trafficking, its binding to blocking drugs and α1 gating features (Triggle, 2006). LTCC are found in smooth and skeletal muscle, ventricular myocytes and osteoblasts, but its presence has also been seen in non-excitable tissues and tumours, highlighting the possible relationship between these channels and cell's malignant processes (Catterall, 2011; Wang et al., 2015).

$Ca_V1.1$ gene expression was increased in acute myeloid leukaemia, in brain desmoplastic medulloblastoma and primitive neuroectodermal tumours, when compared with non-tumoral tissue samples. $Ca_V1.2$ and $Ca_V1.3$ were also overexpressed in several tumours, including breast carcinomas. Only $Ca_V1.4$, which are physiologically found in the retina, have not yet been identified in carcinomas (Wang et al., 2015). Some studies demonstrate that LTCC-mediated Ca^{2+} influx has a role in increasing migration of breast cancer cell lines, MDA-MB-468 (Garib et al., 2005), as well as regulating the filopodia and tumour invasion in MDA-MB-231, PDAC and U2OS (Jacquemet et al., 2016). In addition, it has already been shown that $Ca_V1.2$ expression in MCF-7 cells can be regulated by dihydrotestosterone and results in decreased proliferation (Marques et al., 2015), and also that LTCC can mediate proliferation in HepG2.2.15 (Phiwchai et al., 2020). Its role in apoptosis and even modulating drug resistance in colon tumours and diffuse large B cell lymphomas, respectively, has been reported (Zawadzki et al., 2008; Zhang et al., 2019).

LTCC may be related mainly to migration and proliferation of some tumours, including the one with the highest incidence and mortality among women worldwide, breast carcinoma (Wild et al., 2020). Invasive breast cancer of no special type (NST) is the most common histological classification of breast cancer (Bandyopadhyay et al., 2018). Its neoplastic transformation model starts with ductal hyperplasias (DH), which can transform to ductal carcinoma *in situ* (DCIS) and, finally, in NST. These proliferative alterations are considered possible non-obligate precursors of NST (Lopez-Garcia et al., 2010; Simpson et al., 2005) and women with one of these diagnoses is two to ten times more likely to develop invasive lesions (Carraro et al., 2014; Dupont and Page, 1985; Hartmann et al., 2014; Menes et al., 2017).

It is well established that increased risk pre-neoplastic lesions transformation is related to high proliferative rates (Santisteban et al., 2010; Shaaban et al., 2002) and although there are suggestions of LTCC involvement in breast tumour cells by modulating proliferation and migration, no studies have evaluated whether these channels could participate in tumour transformation process for NST. Therefore, this work aim is to analyse protein expression and subcellular localization of $Ca_V1.1$, $Ca_V1.2$ and $Ca_V1.3$ in human breast tissues without alteration and in proliferative, non-neoplastic and neoplastic ductal epithelial lesions. These analyses support the hypothesis of LTCC participation in breast cancer neoplastic transformation from pre-neoplastic lesions, mainly related to changes in their localization in the mammary epithelium.

Methods

Specimens selection and histopathological evaluation

Human mammary gland tissues, stored in paraffin blocks, from 30 patients from *Hospital Santa Casa de Misericórdia in Belo Horizonte*, between 2010 and 2015, were reviewed for their histological classification and material quality prior of immunohistochemistry technique. The study was approved by the local hospital Ethics Committee, with adherence to the Declaration of Helsinki. The approval ID of the Ethics Committee is 33481919.4.0000.5149 and 33481919.4.3001.5138.

Histological analyses were carried out using Hematoxylin-Eosin staining and optical microscopy, and were used to classify, according to World Health Organization guidelines (Tan et al., 2019), different regions of each sample in Breast without alteration (BWA), Ductal hyperplasia, Ductal carcinoma *in situ* and Invasive breast cancer of no special type (NST). To allow future statistical analysis, the classified regions received scores 1, 2, 3 and 4, respectively. Reinforcing the idea that each sample analyzed can have different regions with different histological alterations, in addition to the normal mammary epithelium, the regions were classified and analyzed individually.

Immunohistochemistry

To perform the immunohistochemistry technique, 4 μm histological sections of samples were obtained, mounted on gelatinized slides, deparaffinized and rehydrated using graded concentrations of ethanol. The immunohistochemistry staining was carried out using the peroxidase reaction with identification from the polymerized secondary antibody (Novocastra Post Primary and Novolink Polymer; Leica Biosystems, Newcastle upon Tyne, UK). The antigen retrieval occurred in pressurized humid heat at 137°C (Autoclave ALT 5LD plus; ALT, São Paulo, Brazil) with Target Retrieval Solution Citrate - low pH (Agilent Technologies, California, US). To block endogenous peroxidase and proteins, the slides were incubated in Novocastra Peroxidase Block and Novocastra Protein

Block (Leica Biosystems, Newcastle upon Tyne, UK). Antibodies against $Ca_V1.1$ (G-1, sc-514685), $Ca_V1.2$ (D-6, sc-398433) and $Ca_V1.3$ (E-3, sc-515679), all from Santa Cruz Biotechnology, were used at concentrations of 1:50, 1:50 and 1: 150, respectively. The incubation time for the primary antibodies was 16 hours and for the 3'3-diaminobenzidine (DAB) chromogen (Leica Biosystems, Newcastle upon Tyne, UK), 1 minute. After DAB incubation, the sections were counterstained with Hematoxylin. Samples of human skeletal muscle for $Ca_V1.1$, human smooth muscle for $Ca_V1.2$ and $Ca_V1.3$ were used for positive control. For negative control, primary antibody incubation stage was replaced by incubation with mouse immunoglobulins, which is the species that the primary antibodies were produced.

Interpretation of immunohistochemical findings

All histological analyzes were performed using conventional optical microscopy in a 40x objective (Olympus - BX41) and the images were captured from a system using a SPOT 3.4.5 Basic® camera adapted to an Olympus Microscope (BX41). Some samples that did not show immunohistochemical reactivity for certain antibodies were discarded.

Each protein had its cytoplasmic, nuclear and membrane immunostaining analyzed for all studied regions. Cytoplasmic expression was assessed according to the immunostaing intensity and followed the score: 0: the absence of expression; 1+: weak; 2+: moderate; 3+: strong. For the nuclear expression analysis, each area had its cells percentage first estimated in a semi-quantitative way, being classified with no expression, weak, moderate or strong intensity. Then, these data were segregated into negative expression, considering the sum of regions with no expression and weak intensity, and positive expression, with this being the samples with moderate or strong intensity. The percentages of nuclear expression were also classified according to the predominant expression intensity, where: 0: the absence of expression; 1+: weak intensity; 2+: moderate; 3+: strong. Membrane expression, on the other hand, was classified by the percentage of cells that had this immunostaining and followed score 0 (absence of expression); 1+ (<25%); 2+ (25% - 50%); 3+ (> 50%).

Statistical analysis

Possible correlations were assessed using the Pearson test. Results were considered significant when the probability of test significance was $p < 0.05$. Statistical analyzes were performed with the aid of GraphPad Prism v. 8.0 (GraphPad Software, La Jolla, CA, USA).

Results

Histopathological analyzes were performed in 30 cases and different regions of the same sample were analyzed and classified individually. From this analysis, 44 distinct histological aspects were identified and in 11 cases there was more than one region to be analyzed. With 13 histological areas with BWA and 15 with DH, only one of the DH regions was associated with neoplastic lesions. Also, 9 with DCIS and 7 with NST were analysed. $Ca_V1.1$, $Ca_V1.2$ and $Ca_V1.3$ immunohistochemistry was performed in all cases and the immunoreaction was analyzed for nuclear, cytoplasmic and membrane immunostaining (Fig. 1).

The immunostaining analysis for $Ca_V1.3$ presented a negative correlation between its nuclear expression and the histological classification of the lesions ($r = -0.43$; $p < 0.05$; Table 1), indicating that there is a reduction in this protein expression as the cells advance in the stepped transformation model for the NST. $Ca_V1.1$ showed a higher expression in DH but with no statistical difference in correlation analysis with histological classification. Differently, $Ca_V1.2$ showed weak or absent nuclear expression in all analyzed cases, which were categorized as negative nuclear expression.

In cytoplasmic immunostaining analysis, we observed protein expression for all three channels and surprisingly, the cytoplasmic immunostaining of $Ca_V1.1$ showed a positive correlation with the predominant nuclear expression score ($r = 0.62$; $p < 0.05$; Table 2). This result shows that this isoform follows the same pattern of immunoexpression in different cell compartments, whether in high or low expression. Regarding the cytoplasmic expression of $Ca_V1.2$, 100.0% of the samples presented cytoplasmic marking, as well as 100.0% of NST cases were positive for $Ca_V1.3$ immunostaining, not being possible to observe correlations with the nuclear expression pattern Table 3

Membrane immunoexpression was observed only for $Ca_V1.2$ and 1.3, with a gradual increase in immunoexpression being revealed in hyperplastic and neoplastic lesions Table 4. This profile of membrane immunostaining was corroborated by the analysis of the positive correlation between histological classification and membrane expression score for $Ca_V1.2$ ($r = 0.69$; $p < 0.05$) and $Ca_V1.3$ ($r = 0.54$; $p < 0.05$; Table 1), showing that there is an increase in this expression as the cells advance in the model of progression in stages for the NST.

Discussion

In summary, our results showed a higher nuclear expression of $Ca_V1.3$ in lesions at the beginning of the neoplastic transformation model, which may suggest that the role played by this channel in the nucleus of breast epithelial

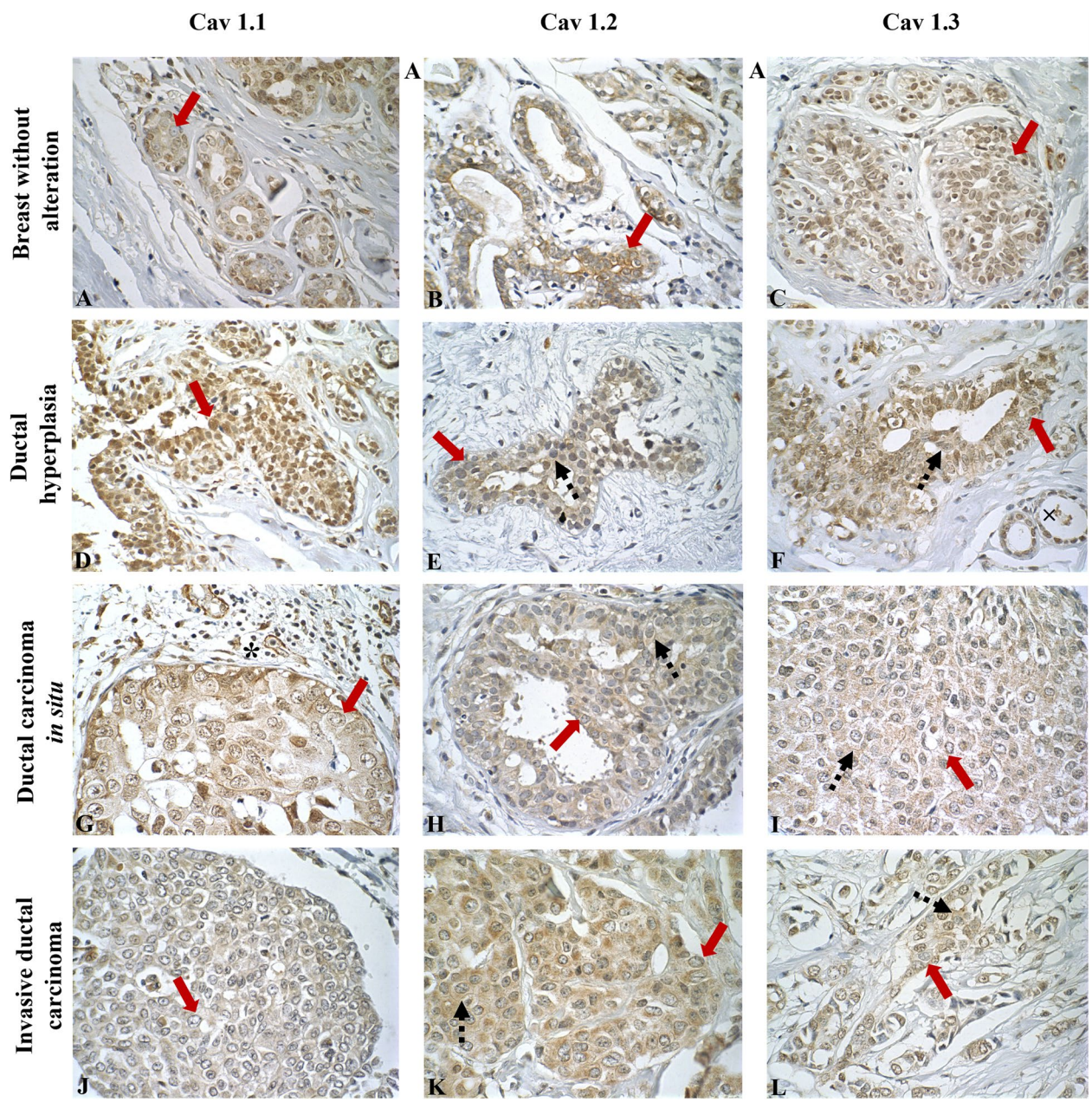

Fig 1. Human mammary glands with immunostaining for $Ca_V1.1$, $Ca_V1.2$ and $Ca_V1.3$. Identified by DAB chromogen and counterstained with hematoxylin. 600x magnification. **A**-**C** mammary lobes without histological alterations. **D**-**F** ductal regions with three or more layers of epithelial cells above the basal membrane and presence of a ductal region without alteration in F (×). **G**-**I** epithelial cell proliferation forming intraductal projections or solid masses restricted to the terminal ductal lobular units with an inflammatory infiltrate associated in G (*). **J**-**L** atypical epithelial proliferation, creating isolated cell nests in the mammary stroma

cells may be related, in a still unknown mechanism, with the neoplastic transformation process. Also, it was seen that $Ca_V1.2$ and $Ca_V1.3$ showed an increase in membrane expression as the regions advanced in the histological classifications, indicating a possible role of these isoforms in the transformation process from non-neoplastic proliferative epithelial lesions of the breast to neoplastic lesions.

So far, LTCC presence in cell nucleus has not been studied, but the presence of a variety of ion channels in nuclear membrane, such as Ryanodine and Inositol triphosphate receptors, is already known. In addition,

Table 1 Pearson correlation coefficient matrix for $Ca_V1.3$ immunohistochemical staining correlating all the analysed parameters. Histological classification was correlated positivity with membrane immunostaining and negativity correlated with nuclear immunostaining

	Cytoplasmic immunostaining	Nuclear immunostaining positive/negative	Predominant nuclear immunostaining	Histological classification	Membrane immunostaining
Cytoplasmic immunostaining	1.00	--	--	--	--
Nuclear immunostaining positive/negative	0.15	1.00	--	--	--
Predominant nuclear immunostaining	0.16	0.68	1.00	--	--
Histological classification	0.07	-0.15	-0.43 *	1.00	--
Membrane immunostaining	0.00	0.00	-0.13	0.54 *	1.00

* indicate $p < 0.05$

Table 2 Pearson correlation coefficient matrix of $Ca_V1.1$ immunohistochemical staining correlating all the analysed parameters. Cytoplasmic immunostaining was correlated positively with nuclear immunostaining

	Cytoplasmic immunostaining	Nuclear immunostaining positive/negative	Predominant nuclear immunostaining	Histological classification
Cytoplasmic immunostaining	1.00	--	--	--
Nuclear immunostaining positive/negative	0.40 *	1.00	--	--
Predominant nuclear immunostaining	0.62 *	--	1.00	--
Histological classification	-0.08	-0.15	-0.31	1.00

* indicate $p < 0.05$

Table 3 Percentage of cases with cytoplasmic immunostaining categorized in no expression, and weak, moderate, or strong intensity of $Ca_V1.1$, $Ca_V1.2$ and $Ca_V1.3$ immunostaining in all analysed regions. Cytoplasmic immunoexpression is seen for all channels in most cases of all regions

			No expression	Weak	Moderate	Strong
CYTOPLASMIC	1.1	Breast without alteration	18.1% (2/11)	45.4% (5/11)	36.3% (4/11)	0.0% (0/11)
		Ductal hyperplasia	10.0% (1/10)	50.0% (5/10)	40.0% (4/10)	0.0% (0/10)
		Ductal carcinoma *in situ*	33.3% (1/3)	66.6% (2/3)	0.0% (0/3)	0.0% (0/3)
		Invasive breast carcinoma of no special type	16.6% (1/6)	66.6% (4/6)	16.6% (1/6)	0.0% (0/6)
	1.2	Breast without alteration	0.0% (0/10)	60% (6/10)	40% (4/10)	0.0% (0/10)
		Ductal hyperplasia	0.0% (0/11)	63.6% (7/11)	27.2% (3/11)	9.0% (1/11)
		Ductal carcinoma *in situ*	0.0% (0/5)	80.0% (4/5)	20.0% (1/5)	0.0% (0/5)
		Invasive breast carcinoma of no special type	0.0% (0/3)	33.3% (1/3)	66.6% (2/3)	0.0% (0/3)
	13	Breast without alteration	9.0% (1/11)	36.3% (4/11)	45.4% (5/11)	9.0% (1/11)
		Ductal hyperplasia	8.3% (1/12)	66.6% (8/12)	25% (3/12)	0.0% (0/12)
		Ductal carcinoma *in situ*	33.3% (2/6)	50.0% (3/6)	16.3% (1/6)	0.0% (0/6)
		Invasive breast carcinoma of no special type	0.0% (0/6)	50.0% (3/6)	50.0% (3/6)	0.0% (0/6)

presence of another family of voltage-dependent Ca^{2+} channels, the R-type channels, has been described. They have already been observed in the nucleus of human aorta epithelial cells and other diverse cell types. Once in the nucleus membrane, these channels have a role in nuclear Ca^{2+} homeostasis with a sustained Ca^{2+} current, being important in gene expression, protein synthesis, as well as macromolecules trafficking through the nuclear pore complexes (Bkaily et al., 2009). The decrease in LTCC expression in the nucleus of neoplastic cells may be related to the deregulation of some cellular processes, contributing to tumour transformation.

The presence of abundant cytoplasmic immunostaining in all histological classifications analyzed suggests that these channels play essential functions in intracellular organelles. The traffic between the endoplasmic

Table 4 Percentage of cases with membranar immunostaining categorized in no expression, <25%, 25% a 50%, or <50% of $Ca_V1.2$ and $Ca_V1.3$ immunostaining in all analyzed regions. Membrane immunoexpression increase in hyperplastic and neoplastic lesions

			No expression	(<25%)	(25% - 50%)	(>50%)
MEMBRANAR	1.2	Breast without alteration	100.0% (10/10)	0.0% (0/10)	0.0% (0/10)	0.0% (0/10)
		Ductal hyperplasia	45.4% (5/11)	36.3% (4/11)	0.0% (0/11)	18.1% (2/11)
		Ductal carcinoma *in situ*	0.0% (0/5)	40.0% (2/5)	40.0% (2/5)	20.0% (1/5)
		Invasive breast carcinoma of no special type	0.0% (0/3)	0.0% (0/3)	100% (3/3)	0.0% (0/3)
	1.3	Breast without alteration	100.0% (11/11)	0.0% (0/11)	0.0% (0/11)	0.0% (0/11)
		Ductal hyperplasia	66.6% (8/12)	8.3% (1/12)	8.3% (1/12)	16.6% (2/12)
		Ductal carcinoma *in situ*	50.0% (3/6)	16.6% (1/6)	16.6% (1/6)	16.6% (1/6)
		Invasive breast carcinoma of no special type	16.6% (1/6)	16.6% (1/6)	33.3% (2/6)	33.3% (2/6)

reticulum (where they are produced) and cellular or organelles membrane aims to regulate not only the function but also the channels expression, being β and α2δ subunits responsible for protein addressing control (Dolphin, 2018; Weiss and Zamponi, 2017). In cytoplasmic organelles, lysosomes are one of the possible localizations of LTCC, since voltage-gated Ca^{2+} channels presence has already been seen in these structures (Zhong et al., 2017). Once in lysosomes, Ca^{2+} ions influx can modulate autophagy, an important process for the survival of tumour cells (Kondratskyi et al., 2013). In addition to lysosomes, another possible localization would be the mitochondria and endoplasmic reticulum, where the presence of other ion channels related to apoptosis inhibition of tumour cells has been reported (Brini, 2000; Leanza et al., 2013).

Once in the cellular membrane, LTCC can contribute for Ca^{2+} homeostasis and, consequently, the control of different cellular processes. An example is the LTCC-mediated Ca^{2+} entry, that can trigger the activation of different transcription factors, such as CREB, NFAT and DREAM that will act on gene regulation (Barbado et al., 2009) and may be responsible for tumour progression of cells seen in this study. CREB is usually overexpressed in breast carcinomas and is associated with resistance to antiproliferative signals, apoptosis, angiogenesis, invasion and metastasis (Steven et al., 2020). Also, it has been seen that increasing the Ca^{2+} intracellular concentration through $Ca_V1.2$ leads to cleavage and nucleus translocation of a C-terminus fragment of the pore-forming α1 subunit, called CCAT, that can bind to transcriptional regulators gene and thus modulate the expression of several genes (Barbado et al., 2009). Besides, the absence of immunostaining for $Ca_V1.1$ indicates that this channel may be involved in functions other than Ca^{2+} influx at the plasma membrane (Arcangeli et al., 2008; Capiod, 2011).

One of the limiting factors of this work was a small sample size, which made it impossible for further stratifications in analyzed groups. More cases would allow L-type Ca^{2+} channels expression analysis in usual and atypical ductal hyperplasias or carcinomas with different histological grades, for example. Furthermore, the impossibility of accessing clinical data from patients should also be considered a limitation. Since the investigation of L-type channels in neoplastic and pre-neoplastic lesions of the human breast was carried out by this work, new studies may emerge to further investigate the role of LTCC in each one of the lesions mentioned.

Conclusions

In conclusion, although there is still no evidence in the mechanisms, these results suggest a role for $Ca_V1.1$, $Ca_V1.2$ and $Ca_V1.3$ in the breast tumour transformation process. Therefore, a better understanding of the role that each of the isoforms is playing in different subcellular localization and the traffic dynamics of these channels and the analysis of a greater number of cases can allow a better understanding of neoplastic transformation and allow to explore these channels in treatment, with the use of existing LTCC blocking drugs, or even as a biomarker molecule that can be used in neoplastic diagnosis or prognosis.

Abbreviations

BWA: Breast without alteration; DCIS: ductal carcinoma *in situ*; DH: ductal hyperplasias; LTCC: L-type Ca^{2+} channels; NST: Invasive breast cancer of no special type; DAB: 3'3-diaminobenzidine.

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Authors' contributions
Conception and study design: F.A., A.L.R. and E.F.; case selection: F.A., E.B; E.F.; sample processing and experiments: F.A.; data analysis and interpretation: F.A.; P.R.; A.L.R.; E.F.; wrote the manuscript: F.A.; E.F.. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interest
The authors declare that they have no competing interests.

Ethics approval and consent to participate
This work was carried following the ethical principles for the use of human material as a primary source of information after approval by the Research Ethics Committee (Comitê de Ética em Pesquisa – COEP) of the Federal University of Minas Gerais and of the Hospital Santa Casa de Misericordia de Belo Horizonte (33481919.4.0000.5149 and 33481919.4.3001.5138, respectively). All authors consent to participate.

Consent for publication
Not applicable

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References

Arcangeli A, Crociani O, Lastraioli E, Masi A, Pillozzi S, Becchetti A. Targeting Ion Channels in Cancer: A Novel Frontier in Antineoplastic Therapy. Curr Med Chem. 2008. https://doi.org/10.2174/092986709787002835.
Bandyopadhyay S, Bluth MH, Ali-Fehmi R. Breast Carcinoma: Updates in Molecular Profiling 2018. Clin Lab Med. 2018. https://doi.org/10.1016/j.cll.2018.02.006.
Barbado M, Fablet K, Ronjat M, De Waard M. Gene regulation by voltage-dependent calcium channels. Biochim Biophys Acta - Mol Cell Res. 2009. https://doi.org/10.1016/j.bbamcr.2009.02.004.
Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: Dynamics, homeostasis and remodelling. Nat Rev Mol Cell Biol. 2003. https://doi.org/10.1038/nrm1155.
Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol. 2000. https://doi.org/10.1038/35036035.
Bkaily G, Avedanian L, Jacques D. Nuclear membrane receptors and channels as targets for drug development in cardiovascular diseases. Can J Physiol Pharmacol. 2009. https://doi.org/10.1139/Y08-115.
Brini M. Effects of PMCA and SERCA pump overexpression on the kinetics of cell Ca2+ signalling. EMBO J. 2000. https://doi.org/10.1093/emboj/19.18.4926.
Capiod T. Cell proliferation, calcium influx and calcium channels. Biochimie. 2011. https://doi.org/10.1016/j.biochi.2011.07.015.
Carraro DM, Elias EV, Andrade VP. Ductal carcinoma in situ of the breast: Morphological and molecular features implicated in progression. Biosci Rep. 2014. https://doi.org/10.1042/BSR20130077.
Catterall WA. Voltage-Gated Calcium Channels. Cold Spring Harb Perspect Biol. 2011. https://doi.org/10.1101/cshperspect.a003947.
Catterall WA, Perez-Reyes E, Snutch TP, Striennssnig J. International Union of Pharmacology. XLIX. Nomenclature and structure-function relationships of transient receptor potential channels. Pharmacol Ver. 2005. https://doi.org/10.1124/pr.57.4.6.
Déliot N, Constantin B. Plasma membrane calcium channels in cancer: Alterations and consequences for cell proliferation and migration. Biochim Biophys Acta - Biomembr. 2015. https://doi.org/10.1016/j.bbamem.2015.06.009.
Dolphin AC. Voltage-gated calcium channel α2δ subunits: an assessment of proposed novel roles. F1000Research. 2018. https://doi.org/10.12688/f1000research.16104.1.
Dupont WD, Page DL. Risk Factors for Breast Cancer in Women with Proliferative Breast Disease. N Engl J Med. 1985. https://doi.org/10.1056/NEJM198501173120303.
Garib V, Lang K, Niggemann B, Z KS, Brandt L, Dittmar T. Propofol-induced calcium signalling and actin reorganization within breast carcinoma cells. Eur J Anaesthesiol. 2005. https://doi.org/10.1017/s0265021500500102x.
Hartmann LC, Radisky DC, Frost MH, Santen RJ, Vierkant RA, Benetti LL, et al. Understanding the Premalignant Potential of Atypical Hyperplasia through Its Natural History: A Longitudinal Cohort Study. Cancer Prev Res. 2014. https://doi.org/10.1158/1940-6207.CAPR-13-0222.
Jacquemet G, Baghirov H, Georgiadou M, Sihto H, Peuhu E, Cettour-janet P, et al. L-type calcium channels regulate filopodia stability and cancer cell invasion downstream of integrin signalling. Nat Commun. 2016. https://doi.org/10.1038/ncomms13297.
Kondratskyi A, Yassine M, Kondratska K, Skryma R, Slomianny C, Prevarskaya N. Calcium-permeable ion channels in control of autophagy and cancer. Front Physiol. 2013. https://doi.org/10.3389/fphys.2013.00272.
Leanza L, Biasutto L, Managò A, Gulbins E, Zoratti M, Szabò I. Intracellular ion channels and cancer. Front Physiol. 2013. https://doi.org/10.3389/fphys.2013.00227.
Lopez-Garcia MA, Geyer FC, Lacroix-Triki M, Marchió C, Reis-Filho JS. Breast cancer precursors revisited: Molecular features and progression pathways. Histopathology. 2010. https://doi.org/10.1111/j.1365-2559.2010.03568.x.
Marques R, Peres C, Vaz C, Gomes I, Figueira M, Cairrao E, et al. 5 a -Dihydrotestosterone regulates the expression of L-type calcium channels and calcium-binding protein regucalcin in human breast cancer cells with suppression of cell growth. Med Oncol. 2015. https://doi.org/10.1007/s12032-015-0676-x.
Menes TS, Kerlikowske K, Lange J, Jaffer S, Rosenberg R, Miglioretti DL. Subsequent Breast Cancer Risk Following Diagnosis of Atypical Ductal Hyperplasia on Needle Biopsy. JAMA Oncol. 2017. https://doi.org/10.1001/jamaoncol.2016.3022.
Phiwchai I, Thongtem T, Thongtem S, Pilapong C. Liver Cancer Cells Uptake Labile Iron via L-type Calcium Channel to Facilitate the Cancer Cell Proliferation. Cell Biochem Biophys. 2020. https://doi.org/10.1007/s12013-020-00951-0.
Santisteban M, Reynolds C, Barr Fritcher EG, Frost MH, Vierkant RA, Anderson SS, et al. Ki67: a time-varying biomarker of risk of breast cancer in atypical hyperplasia. Breast Cancer Res Treat. 2010. https://doi.org/10.1007/s10549-009-0534-7.
Shaaban AM, Sloane JP, West CR, Foster CS. Breast cancer risk in usual ductal hyperplasia is defined by estrogen receptor-α and Ki-67 expression. Am J Pathol. 2002. https://doi.org/10.1016/S0002-9440(10)64879-1.
Simpson PT, Reis-Filho JS, Gale T, Lakhani SR. Molecular evolution of breast cancer. J Pathol. 2005. https://doi.org/10.1002/path.1691.
Steven A, Friedrich M, Jank P, Heimer N, Budczies J, Denkert C, et al. What turns CREB on? And off? And why does it matter? Cell Mol Life Sci. 2020. https://doi.org/10.1007/s00018-020-03525-8.
Tan PH, Ellis I, Allison K, Brogi E, Fox SB, Lakhani S, et al. The 2019 World Health Organization classification of tumours of the breast. Histopathology. 2020; https://doi.org/10.1111/his.14091.
Triggle DJ. L-Type Calcium Channels. Curr Pharm Des. 2006. https://doi.org/10.2174/138161206775474503.

Wang CY, Lai MD, Phan NN, Sun Z, Lin YC. Meta-analysis of public microarray datasets reveals voltage-gated calcium gene signatures in clinical cancer patients. PLoS One. 2015. https://doi.org/10.1371/journal.pone.0125766.
Weiss N, Zamponi GW. Trafficking of neuronal calcium channels. Neuronal Signal. 2017. https://doi.org/10.1042/ns20160003.
Wild C, Weiderpass E, Stewart B. World cancer report: Cancer Research for. Cancer Prevention. 2020.
Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol Ver. 2015. https://doi.org/10.1124/pr.114.009654.
Zawadzki OA, Liu MDQ, Wang PDY, Ph D, Melander CA, Ph D, et al. Verapamil Inhibits L-type Calcium Channel Mediated Apoptosis in Human Colon. Cancer Cells. 2008. https://doi.org/10.1007/s10350-008-9372-7.
Zhang J, Zhang P, Zhou W, Yu J, Yao Z, Chu J, et al. L-Type Cav 1. 2 Calcium Channel- a -1C Regulates Response to Rituximab in Diffuse Large B-Cell Lymphoma. Clin Cancer Res. 2019. https://doi.org/10.1158/1078-0432.CCR-18-2146.
Zhong XZ, Yang Y, Sun X, Dong XP. Methods for monitoring Ca2+ and ion channels in the lysosome. Cell Calcium. 2017. https://doi.org/10.1016/j.ceca.2016.12.001.

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