INTRODUCTION

The human calreticulin gene (CALR) is located on chromosome 19p13.2 with nine exons. Calreticulin promoter region contains several binding sites for reputed transcription factors and many of these factors have been identified as important modulators of CRT expression including NKx2.5, MEF2C, COUP-TF1, GATA6, Evi-1, and PPAR factors. Moreover, calcium (Ca++) depletion and endoplasmatic reticulum (ER) stress were shown to be essential activators of CALR transcription. Recently, studies have also demonstrated that nerve growth factor (NGF) can also upregulate CALR expression in both ovarian cells and neuronal differentiation. These results suggested an involvement of CALR expression in various biological and pathological processes (Table I).

From several years, many studies have shown that calreticulin is involved in the development of different cancers concerning tumor generation and progression. On the other hand, CALR expressed on cell surface represent a phagocytic signal for natural killer cells by immune system. In addition to immunogenicity and tumorigenesis, interactions throughout cell adhesion between CALR and integrins indicate the primary role of the CALR in cancer metastasis.

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evidence that modification of CALR levels affects cell adhesion on extracellular matrix molecules (ECM). In fact, is noted that CALR plays a role in the control of cell adhesiveness through regulation of fibronectin expressions and collagen deposition mediated by Ca^{2+} regulation and c-SRC activity. In addition, previous studies revealed that CALR-mediated cell adhesion might be due to direct interaction between CALR and integrins by binding to the cytoplasmic KGFFKR motif of the integrin α-subunit. These studies provided evidence that CALR plays a pivotal role in cellular adhesiveness.

Here, we reviewed the biological roles of CALR in cancer development. Also, available methods for low detection amount of mutant DNA in a large excess of wild type (wt) DNA are taken in consideration.

**Mechanisms of Cancer development involving CALR**

The cell adhesion is based on the regulation of focal contact via multiple mechanisms and the hypothesis that CALR might be involved is based on evidence that modification of CALR levels affects cell adhesion on extracellular matrix molecules (ECM). In fact, is noted that CALR plays a role in the control of cell adhesiveness through regulation of fibronectin expressions and collagen deposition mediated by Ca^{2+} regulation and c-SRC activity. In addition, previous studies revealed that CALR-mediated cell adhesion might be due to direct interaction between CALR and integrins by binding to the cytoplasmic KGFFKR motif of the integrin α-subunit. These studies provided evidence that CALR plays a pivotal role in cellular adhesiveness.
Several studies have demonstrated that CALR expression levels were positively correlated with tumorigenesis and most of them have indicated that tumor tissues express significantly higher levels of CALR in comparison to standard tissues. Important correlations with clinical stages were found in gastric, breast, and prostate cancer. Other studies also revealed CALR expression levels to be significantly upregulated in oral cancer. Also, there are diverse indications that increased CALR expression might play a critical role during cancer progression. It is found that the concentration of urinary CALR has a tendency to increase in high-grade tumors, and was postulated the use of CALR dosage as a biomarker for urothelial cancer.

However, low levels of CALR were observed in malignant effusions of high-grade ovarian carcinoma but the roles of CALR in ovarian cancer progression are open to doubt.

Finally, CALR expression levels may be statistically linked with better response to chemotherapy while the survival rate was not correlated to CALR. Furthermore, in neuroblastoma, enhanced CALR expression is related with tissues histotype.

**CALR mutations as marker for mielodysplastic syndrome**

Mutations in CALR gene were detected preferentially in exon 9 including 52 bp deletion and 5 bp insertion of individual base pairs, which leads to frame shift mutations. These mutations were strongly correlated to myeloproliferative neoplasms (MPN), including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). It is noted that patients with MPN (95% of PV, 60% ET and about 25% PMF) were found to have mutations in exon 14 of Janus kinase 2 gene (JAK2) at codon V617. For the remaining patients, others mutations in JAK2 exon 12 (<5%), in MPL gene codon W515K/L were identified. As a consequence, CALR mutated gene, encoded a protein deleted by the C-terminal KDEL domain; therefore, they may affect normal Ca++ binding and physiological cell growing.

**Methods for detection of known mutation in a large excess of wild type DNA**

The choice of a specific genotyping detection assay for identification of mutations is strongly dependent on the type of mutation and its allele heterogeneity. In general, homogeneous systems increase throughput, reduce the chance of cross contamination and are amenable to automation, but require more fluorescently-labeled probes, increasing costs and reduced multiplexing capabilities. When a large panel of SNPs assays needs to be developed and budget for instruments are limited, methods based on conventional PCR followed by a gel-based detection assay should be preferred over fluorescent hybridization-based methods. Another advantage of electrophoretic detection systems is the possibility to direct check for the appropriate size of amplicons. We define these methods as low-throughput, due to their time-consuming and labor-intensive characteristics. However, fluorescent-based detection systems have been developed for application in laboratories, due to their high specificity, high sensitivity and medium/high-throughput. A comprehensive list of the current methods available for low detection amount of mutant DNA in a large excess of wild type (wt) DNA is summarized in the Table II.

**CONCLUSIONS**

All these new acknowledgments about CALR function in oncology field will allow the design of
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new research project that will get a light to relationship between the inflammation, cellular damage, and tumors development21.

In addition, promising, we believe that the validation of the productive methods able to detect costly either CALR mutations and/or expression allow to integrate this information in a personalized approach22. In this way, in order to interpret correctly lab results of the CALR it is necessary an upgrading of the oncologist in genomics field23.

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CONFLICT OF INTEREST:
None declared
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