World Cancer Research Journal WCRJ 2017; 4 (3): e926

THE EMERGING ROLE OF CALRETICULIN IN CANCER CELLS

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Abstract – *Background:* *Recently, Calreticulin (CALR) has been proposed to participate in various physiological and pathological processes in cells. The two major functions of CALR inside the Endoplasmic Reticulum (ER) are protein chaperoning and regulation of Ca++ homeostasis. Furthermore, accumulated studies indicate that CALR also regulates important biological functions including cell adhesion, gene expression, and RNA stability.*

Upgrading: *This review aims to highlight the most biological function of CALR protein in cancer development. In addition, a comprehensive evaluation of the detection method for revelation of low amount of mutant DNA in a large excess of wild type (wt) DNA is proposed and discussed.*

Conclusions: *Acknowledgments about CALR function in oncology field will allow the design of new research project that will get light to relationship between normal physiological function and tumors development mediated by CALR. Based on these fields, in the next future, the oncologists will have new markers to strike a precise diagnosis.*

KEYWORDS: *CALR, Genomic, Mutation, Tissue expression.*

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[1]. Moreover, calcium (Ca^{++}) depletion and endoplasmatic reticulum (ER) stress were shown to be essential activators of CALR transcription[2]. Recently, studies have also demonstrated that nerve growth factor (NGF) can also upregulate CALR expression in both ovarian cells and neuronal differentiation[3,4]. 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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TABLE 1. Biological function of Calreticulin.

Function	*Action*	*Annotation for cancer*	*Reference*
Protein chaperone	CALR is one of the well characterized lectin-like ER chaperons for many proteins.	CALR taking part in quality control process during protein synthesis, including integrins, surface receptors, and transporters.	[5-9]
Calcium homeostasis	CALR is considered as an intracellular $Ca2^{++}$ regulator because it contains two $Ca2^{++}$-binding sites in the P-domain (high-affinity, low-capacity) and C-domain (low affinity, high-capacity). More than 50% of $Ca2^{++}$ stored in ER lumen associates with CALR.	CALR participate in $Ca2^{++}$ homeostasis.	[10,11]
Cell migration and adhesion	CALR is one of the few cytoplasmic proteins that directly interact with integrin α-subunits. CALR modulates cell adhesion and migration through focal contact dependent manners.	CALR plays a critical role in regulating cell adhesion and migration through various mechanisms.	[12-14]
RNA stability	CARL is a mRNA binding protein that destabilizes type I angiotensin II receptor mRNA by binding to AU-rich region in 3'-UTR. Also, it binds to specific element in 3'-UTR of glucose transporter-1 mRNA and destabilizes the mRNA under high-glucose conditions.	These functions identified a trans-acting factor, which regulates mRNA stability.	[15,16]
Phagocytic signal	Cell surface CALR facilitates the phagocytic uptake of apoptotic and cancer cells. Surface exposure of CALR is required for phagocytosis on dying tumor cells. CALR expressed on the cell surface is considered as an "eat-me" signal for multiple human cancers.	These results indicate that CALR-mediated immune mechanisms might be an important strategy for developing new anticancer therapy.	[17-19]
Cell proliferation	Higher levels of CALR promoted cell proliferation and upregulated the proangiogenic factor vascular endothelial growth factor (VEGF) expression in gastric cancer cells. Prostate cancer cells with higher CALR levels produced fewer colonies as well as inhibition of tumor growth both *in vitro* and *in vivo*. CALR upregulates VEGF expression, suppresses cell proliferation and enhances cell differentiation in neuroblastoma cells.	The effect of CALR on cell proliferation might depend on cell types.	[20-22]

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[5].

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)[6,7]. 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 $Ca2^{++}$- regulation and c-SRC activity[8]. In addition, previous studies[9] revealed that CALR-mediated cell adhesion might be due to direct interaction between CALR and integrins by binding to the cytoplasmic KXGFFKR motif of the integrin α-subunit. These studies provided evidence that CALR plays a pivotal role in cellular adhesiveness.

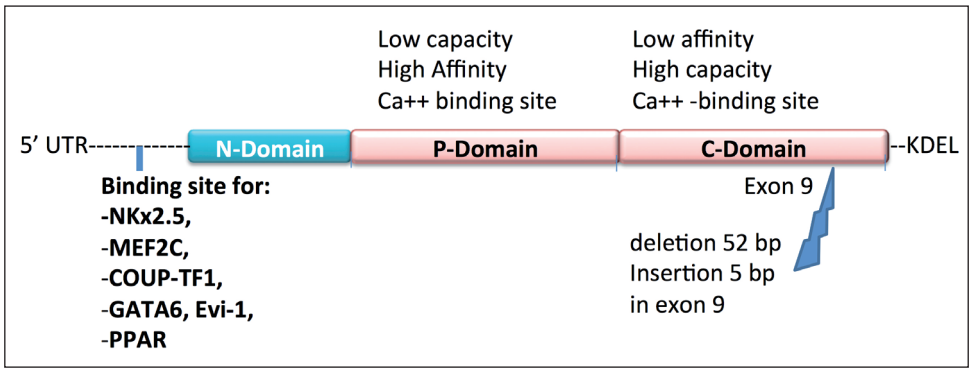

Fig. 1. Schematic representation of CALR.

Several studies[10] 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[11], breast[12], and prostate cancer[13]. Other studies also revealed CALR expression levels to be significantly upregulated in oral cancer[14]. 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 marker[15].

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[16].

Finally, CALR expression levels may be statistically linked with better response to chemotherapy while the survival rate was not correlated to CALR[16,17]. Furthermore, in neuroblastoma, enhanced CALR expression is related with tissues histotype[18].

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[19]. For the remaining patients, others mutations in JAK2 exon 12 (<5%), in MPL gene codon W515K/L were identified[20]. 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 wilt 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[5]. 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

TABLE 2. Comparison of methods for detection of known DNA point mutation in a large excess of Wild type DNA.

Genotyping methods	*Specificity*	*Sensitivity (Mut/Wt)*	*Assay* stability*	*Equipment# required*
ASA	-Medium -High§ (sequencing) 1/100	1/10000	High	Gel electrophoresis system
RFLP	-Medium -High (sequencing)	1/1000 1/10000	Low (restriction enzyme)	Gel electrophoresis system
PNA-mediate	-High	1/100000	High	Gel electrophoresis
PCR calmping	-Very High (Maldi-Tof)	1/1000000		system
FRET allelic discrimination a) **LC probe** b) **TaqMan 5'nuclease (End point detection)** c) **Beacons probe** d) **Scorpions probe Hyb probe**	High	1/100	Middle (probe fluoro-labeled)	Dedicate instrumentation and software
LNA probe	Very High	1/10000	Middle (probe fluoro-lebelled)	Common Fluorescent-detecting instrumentation or plate reader
Invader assay	Very High	1/100	Middle (cleavase enzyme)	Dedicate instrumentation and software
OLA (SNPlex)	Very High	1/100	Middle (probe fluoro-lebelled)	Dedicate instrumentation and software
Pyrosequencing	Very High	1/10000	Middle (luciferase related enzyme)	Pyrosequencer dedicate software
HRM	Medium	1/100	High	Common Fluorescent-detecting

List of Abbreviations: ASA Allelic Specific Assay; RFLP Restriction Fragment Length Polymorphisms; PNA Peptide Nucleic Acid; FRET Fluorescent Resonance Energy Transfer; LNA Locked Nucleic Acid; HRM High Resolution Melt.
§ High/very high specificity, if combined to other detection platform (i.e. sequencer or MALDI-TOF)
* Referred to reagent stability: low = restriction enzyme; Middle = dye-labeled oligonucleotide; High= basic oligonucleotide.
# PCR thermal cycler and other common diffuse instrument, are not included in the estimate equipment.

new research project that will get a light to relationship between the inflammation, cellular damage, and tumors development[21].

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 approach[22]. In this way, in order to interpret correctly lab results of the CALR it is necessary an upgrading of the oncologist in genomics field[23].

Acknowledgements:
The authors wish to thank Dr O. Barletta for bibliography research assistance.

Conflict of interest:
None declared

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