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Proteolysis of Transmembrane Proteins: Degradation or Maturation? Case Study of Disrupted in Renal Carcinoma 2 (DIRC2)

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Re-exporting process of degraded materials from the lumen of lysosomes into cytosol involves lysosomal transmembrane proteins whose defect may lead to mild to severe diseases. Due to the difficulty in the study of transmembrane proteins, our current knowledge in transmembrane proteins, especially in organelle transmembrane proteins are still limited. Disrupted in renal carcinoma 2 (DIRC2), has been recently identified as a constituent of lysosomal transmembrane protein. Structurally, the protein belongs to a member of major facilitator superfamily (MFS), a large group of secondary tranporter proteins with diverse substrates and characterized by the presence of, but not limited to, twelve transmembrane spanning domains. DIRC2 shows an intriguing property that it is fragmented into two nearly equal fragments in the lysosomal fraction of mammalian cells. This study represents the first report of MFS protein internal fragmentation, and a question raised therefore whether the proteolysis lead to degradation or maturation event. Since both fragments were observed in variety of tissues in enormous stability, with exception in cells lacking of cathepsin L, fragmentation of DIRC2 may be regarded as maturation or modification event, instead of a pathway to lysosomal degradation. Whereas the transported substrate by DIRC2 is not known, results shown that DIRC2 in full length form is likely an electrogenic transporter protein of lysosomes.

Keywords: *transembrane protein, renal carsinoma, lysosome*

1. Introduction

The DIRC2 gene was originally reported in the study of renal carcinoma as this gene spans the breakpoint of a chromosomal translocation observed in a rare cases of familial renal cancer in Dutch-Turkish population. *DIRC2* gene is expressed in various tissues, including kidney. *DIRC2* encodes a predicted protein of 478 amino acids with a high homology to the feline leukemia virus subgroup C receptor (FLVCR)[1]. FLVCR involves in the transport of free heme out of cytoplasm, which is other ways toxic for the cells. DIRC2 shows also similarity to various putative transporters of major facilitator superfamily (MFS) transporter proteins. Transcript of the DIRC2 shows a high homology (>80%) to the transcripts from different species, including monkey, pig, dog and mouse. A significant degree of homology (>30-45%) of human DIRC2 to different proteins from *Leishmania major* and *Arabidopsis thaliana* has also emphasized the conservation of this protein[1].

DIRC2 protein is suggested to have 12 transmembrane spanning domains, which is the basic feature of MFS transporter protein. It has also a conserved MFS-specific signature sequence which lays between transmembrane domain 2 and 3 [1]. DIRC2 is one of putative novel lysosomal membrane proteins identified in a recent proteomics study of lysosomes purified from human placenta[2]. Bioinformatic analysis of this DIRC2 suggests that it has one glycosylation site and several phosphorylation sites. Apart from this genetic description of DIRC2, there is currently no further report about the biochemical and molecular aspects of DIRC2.

An immediate finding which build the basis of this study is the fact that DIRC2 is endogenously and heterogenously expressed as fragmented form. Based on the fragmentation pattern, the two asymmetric fragments are, nevertheles, not expected to be the

result of convensional processing of either terminal region. Hence, it is of interest to reveal the uncommon property of this protein.

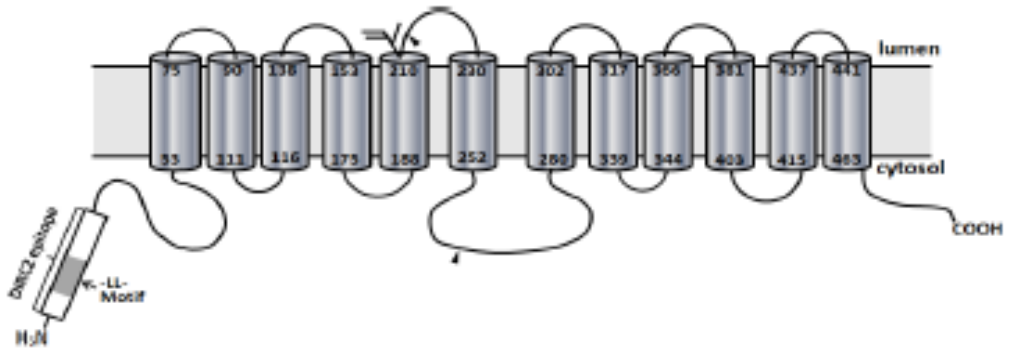

Figure 1. Predicted topology of DIRC2 protein generated by the TMHMM transmembrane protein predictor. DIRC2 consists of twelve transmembrane domain. A dileucine motif is located close to N-terminus and putative glycosylation site is predicted on Asn-209.

2. Methods

C-terminally myc-tagged human DIRC2 was expressed in HeLa cells. Lysate was subjected to Percoll™ fractionation and each fractions were blotted against a line of organelle markers as well as tested for their alkaline phosphatase and beta-hexosaminidase activity. Intracellular fate of DIRC2 was also investigated by using organelle markers and tested by immunofluorescence (not shown). To investigate a possible role of lysosomal proteases in the processing of DIRC2, DIRC2 gene was expressed in MEF (murine embryonic fibroblast) cells deficient in single of double lysosomal proteases.

3. Results and Discussion

By using several biochemical assays, it can be shown that DIRC2 colocalizes to lysosomes. Figure 2 shows that DIRC2 colocalizes in the same fractions with lysosomal membrane markers (B), namely: lysosomal associated membrane protein 2 (LAMP2), cathepsin D (CtsD) and beta-glucocerebrosidase (βGC). It coincidently appears at the same fraction with lysosomal enzyme beta-hexosaminidase activity peak (A). A dileucine motif, known to account for lysosomal localization for many lysosomal membrane proteins, found close to the N-terminus of DIRC2 (Figure 1) also supports lysosomal fate of DIRC2, independent of whether or not its glycosylation site is used (data not shown).

Expression of DIRC2 in several cathepsin deficient MEF cells showed that DIRC2 is fragmented in all cell lines (appeared as full length protein bands of c.a. 55 kDa and fragmented form of 28 kDa), except for that expressed in MEF cell deficient if cathepsin L. This finding underlies the critical role of cathepsin L in the processing of DIRC2 protein. This result does not necessarily imply that cathepsin L directly involve in the processing of DIRC2. Cathepsin L has never been reported to directly cleave internal region of transmembrane protein. It can, however, be involved in the processing of DIRC2 through activation of other, unknown, proteases. Hence, a detailed study to address this matter is demanded in order to elucidate the property of this protein.

An even more significant question is whether the processing of DIRC2 lead to degradation or maturation/modification even. A similar situation for a member of MFS transporter protein, *Lac* Y, has been tested by Weinglass and Kaback who had shown for the first time that an *Escherichia coli* transporter protein can be expressed as two discontinous fragments while retaining its

transport activity[3]. This observation can be understood by the fact that the two fragments may establish interactions through their side chains.

In an initial characterization of DIRC2 function by expression of a dileucine mutant of DIRC2 at the plasma membrane of *Xenopus oocytes* and testing its ability to take up metabolites, it was found that DIRC2 may act as an electrogenic transporter[4]. In this scenario, DIRC2 may partly function to withstand lysosomal cationic substances (Figure 4). Upon identification of the substrate(s) of full length form of DIRC2, it is of interest to analyze the influence of proteolytic processing on the function of DIRC2. A possible interaction between the DIRC2 fragments and the relevance its function could also provide valuable information in order to have a more comprehensive description of this transporter protein.

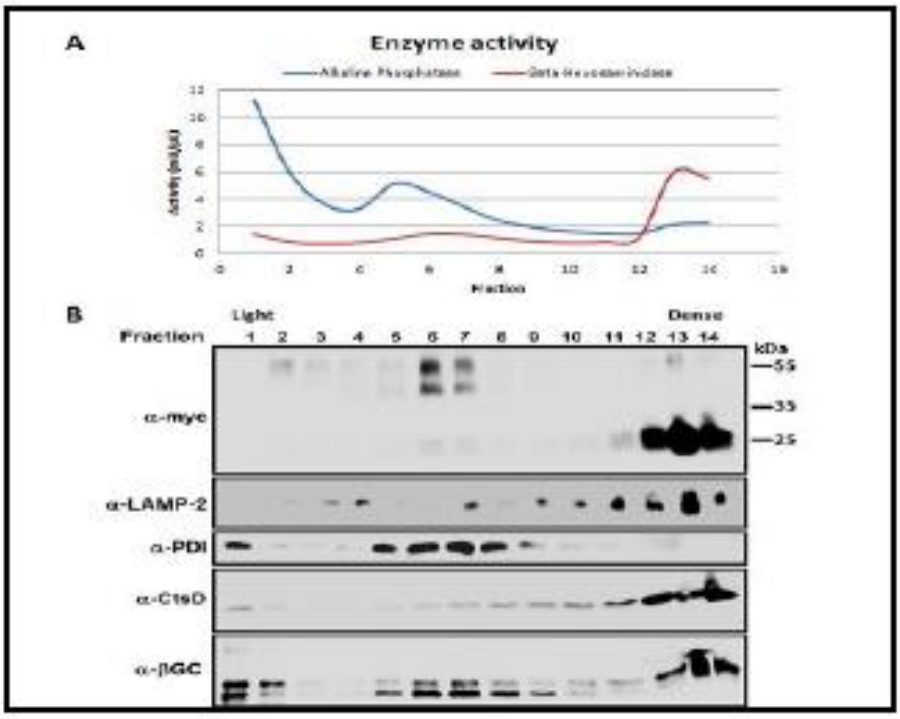

Figure 2. Percoll fractionation of overexpressed DIRC2. Fragmented form of DIRC2 is colocalized in the same fraction as lysosomal markers LAMP2, CtsD, βGC. (LAMP2: lysosomal associated membrane protein 2, PDI: protein disulfide isomerase, CtsD: cathepsin D, βGC: beta glucocerebrosidase)

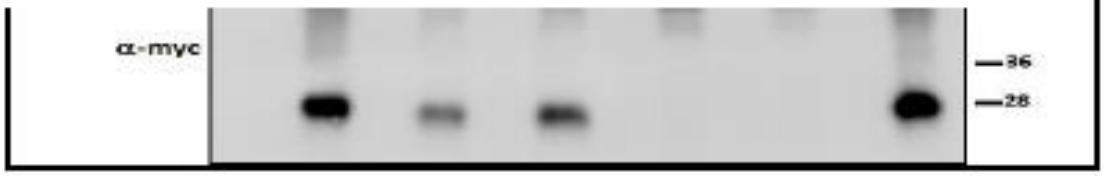

Figure 3. DIRC2 is not fragmented in cathepsin L deficient cell. This underlies the role of cathepsin L in the processing of DIRC2.

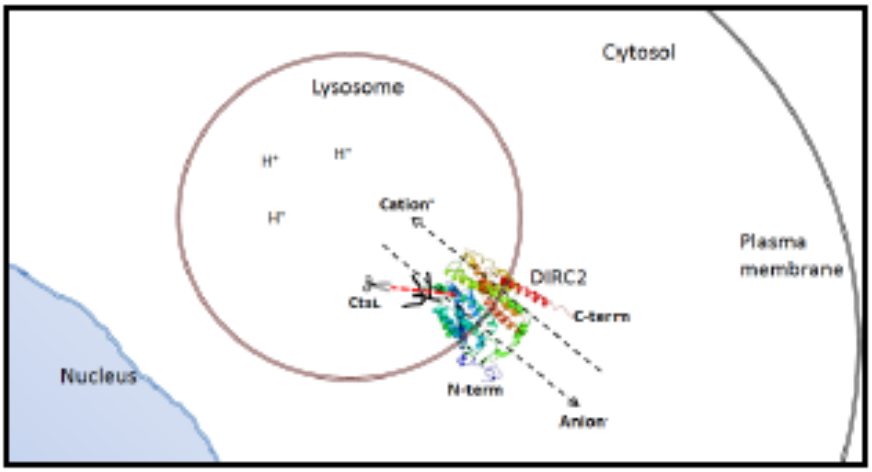

Figure 4. A model proposed for the property of DIRC2 protein. Anion transport from lumen of lysosome is based on electrogenic transport property proposed by Savalas et al[4].

4. Conclusion

It was shown in this study that DIRC2 is a lysosomal membrane protein which is in agreement with bioinformatic analysisof the protein. It is subjected to proteolytic processing within the lysosomes which leads to fragmentation of DIRC2 into two asymmetric fragments. Cathepsin L was shown to play a role in the processing of DIRC2. However, it is not clear whether cathepsin L directly cleaves DIRC2 or indirectly cleaves DIRC2 through activation of another protease(s). It is also not clear whether the processing lead to degradation or maturation pathways. Maturation pathway is no excluded since fragmentation of transporter protein of MFS group may retain its activity[3]. The actual substrate of DIRC2 is yet to be investigated.

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