Comparative Analysis of NLS Sequence Suggests the Evolutionary Origin of Nuclear Matrix Metalloproteinase 7 during Cancer Evolution

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***Abstract*—The exact mechanism of how various proteins translocate from the extracellular into the nucleus to initiate cancer evolution is not known. Specific sequence associated with such nuclear event, thus known as Nuclear Localization Signal (NLS) was investigated. MATRIX Metalloproteinase (MMP) family proteins were found to possess NLS. Research shows over expression of the nuclear MMP(7) protein in different types of human cancer. It is claimed that animals have acquired better pathways to overcome tumor development, therefore it is vital to focus on cancer evolution. The aim of this paper is to investigate evolutionary origin of the NLS in MMP7 by analyzing different species. We found MMP NLS is much conserved but with variations and MMP7 NLS shows the partial consistence with Full-length protein in different species. Our data suggest that nuclear MMP may have undergone evolutionary deviation during natural selection for cancer development.**

***Index Terms*—MMP7, nuclear MMP7, NLS, putative NLS, cancer evolution, cladogram**

I. Introduction

The translocation of different macromolecules happens through their transfer from cytosol into the nucleus [1]. The exact molecular pathway of nuclear transport is unknown. Findings highlight that nuclear proteins carry specific sequence, which signals its proteins to move into the nucleus, known as Nuclear Localization Signal (NLS). Some nuclear proteins control transcription, nucleus organization and mutation or mislocalization may be associated with cancer progression. The origins of NLS should be investigated from the perspective of cancer evolution, since animals have acquired tumor suppression better than humans [2]. Thus, it is crucial to find whether nuclear path of extracellular proteins is due to NLS being old or new event by analyzing various species. Additionally, recent studies identified exact sequence of NLS in Matrix Metalloproteinase (MMP) proteins, which were correlated with cancer and pathological diseases of nervous system [3].

MMP predominately localizes to the extracellular to cleave the matrix for cancer cell invasion. However, recent evidence has shown that MMP also plays essential roles in nucleus. Nuclear MMP9 has been shown to promote rat skeletal myoblasts proliferation [4]. In addition, nuclear MMP12 directly binds NFKBIA promoter and activates its transcription to induce immune response [5]. Early study shows MMP2 translocation in nucleus in endothelial cells and neurons as speckles like structure [6]. MMP2 nuclear localization can be enhanced by irradiation in human glioma xenograft cells [7]. Nuclear MMP2 may interact with many transcription factors such as FOXO4 and GATA-1 [7]. It has been shown that MMP2 and GATA-1 binding promotes IL-10 transcription thereby activating downstream STAT3 signaling for cell migration [7]. Moreover, nuclear MMP3 induces apoptosis through protease activity [8].

The overexpression of nuclear MMP was found in numerous cancer cases. MMP3 has been found in hepatocytes of liver tissues [8] and MMP1 has been shown to be expressed in both breast tumor cells and stromal cells in cancer patient samples [9]. MMP1 expression correlates with cancer grade, HER2 expression and triple-negative cancer types of breast specimens [9]. Although MMP1 alone predicates differential survival value but Ki67 and MMP1 high expression correlates with poor prognostic survival result in breast cancer patients [9]. We recently reported that MMP nuclear localization is correlated to its function in cell migration and cancer progression [10]. In clinical patient samples of prostate cancer, nuclear MMP7 is highly expressed in advanced stage of cancer [10]. Using MMP as a model protein to study the nuclear-extracellular path is of significance in cancer evolution. Therefore, the NLS specifically in MMP(7) within different species will be analyzed.

II. Methods

The PKWRKTH amino acid sequence was the reference for analyzing NLS in different human MMPs obtained from GenBank database [8]. Further, the NLS in human MMP7, PKWTSKV, was the reference for comparison of the NLS in 11 randomly selected species from GenBank database (Fig. 1).

It could be hypothesized that higher the similarity of the whole MMP7 gene in different species to human MMP7, higher similarity of their proteins. Protein BLAST was used to test the hypothesis by obtaining

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percentage of similarity of the full length MMP7 protein in the randomly selected species (Fig. 2).

The second hypothesis states that further common ancestry of different species, the higher the difference of NLS in MMP7. A cladogram, which shows overall picture of organisms' relatedness, but not of individual species, was used to find common ancestor of selected species with human [11]. Thus, the evolutionary origin of nuclear MMP7 was analyzed based on phylogenetic tree (Fig. 3).

Protein interaction maps were constructed by String v10 (http://string-db.org/newstring_cgi/show_input_page.pl?UserId=caTPL_BDN8O4&sessionId=OU1IjN4Eayln&input_page_type=single_sequence).

III. Results

A. Nuclear Localization Signal Exits in MMP Family Proteins

During analyzes of amino acid sequence in various types of MMPs from GenBank database, the sequences similar to the PKWRKTH were found. The overall trend noticed was conservation of the NLS throughout various types of human MMPs (Table I).

TABLE I. The Nuclear Localization Signal in Different Types of Human MMPS

Human MMP	The Nuclear Localization Signal
MMP7	100NS**PKW**TSKVVTYRIVSYTRDL120
MMP9	100 FEGDL**KW**HHHNITYWIQNYSE 120
MMP11	100 SGGR**WEKT**DLTYRILRFPWQL 120
MMP1	105 GNP**R**W**EQTH**LTYRIEN 120
MMP3	105 GI**PKWRKTHL**TYRIVN 120
MMP8	105 N**PKWERT**N**L**TYRIRNY 120
MMP10	105 **PKWRKTHL**TYRIVNYT 120
MMP12	105 GGP**V**W**RK**HYITYRINN 120
MMP13	110 TL**KWSK**MN**L**TY 120
MMP2	115 RKPKWDK 120
MMP14	115 QGL**KW**QH 120
MMP16	120 YALTGQ**KW**QHK 130
MMP17	125 RRQAPAPT**KW**N**K**RN**L**S140
MMP15	130 KRYALTGR**KW**N140

B. Similarity to Human MMP7 NLS in Different Species

Further, the examination of MMP7 in different species for PKWTSKV amino acid sequence also showed the conservation of the NLS (Fig. 1) though similarities are different among different species. It is not surprise that chimpanzee shows the 100% consistence with the human for NLS of MMP7. However, whale and sheep show the 100% consistence with the human for NLS is a big surprise. Moreover, the model animal for human diseases including cancer, mouse, shows only 57% similarity for NLS to human. Thus NLS variation during evolution may be undergone an unexpected jump or branch during evolution.

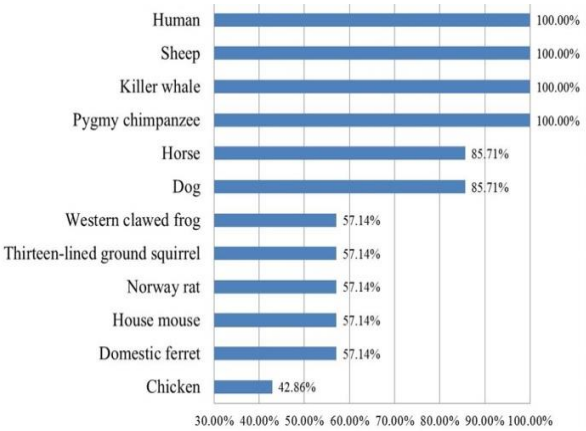

Figure 1. Similarity to human MMP7 NLS in different species.

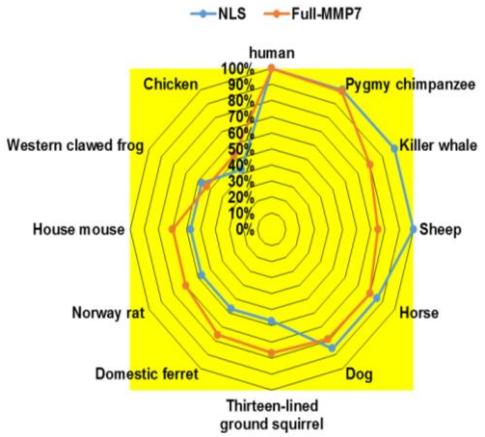

Figure 2. Similarity to human full-MMP7 and NLS in different species.

Additionally, the protein BLAST results showed varying similarity percentage of the full length MMP7 and its corresponding NLS in different species (Fig. 2). As shown in Fig. 2, NLS and full-length MMP7 predominately correlate as far as sequence homologues to human MMP7 is concerned. However, there are exceptions such as dog, horse, sheep and whale. The NLS showed much higher sequence homologues to human MMP7 than that of full-length MMP7.

C. The Evolutionary Origin of MMP7 NLS in Different Species

The evolutionary origin of MMP7 NLS in different species could be investigated based on the created cladogram (Fig. 3). According to cladogram, common ancestor of Western clawed frog, accounting 57.14% similarity with human MMP7 NLS, is Four-Legged (Tetrapod), who evolved during Devonian period about 416 Mya (million years ago) [11]. While, Chicken accounting 42.86% NLS similarity evolved from Amniotic Egg ancestor with human during Carboniferous era 359 Mya. Interestingly, rodent species showed similar percentage of NLS conservation being 57.14%. Even though Western clawed frog possessing ancestry from older period, showed higher NLS similarity than Chicken from closer period, it is not probably indication of old evolutionary origin due to only ½ of nuclear MMP7 being conserved through evolution (Fig. 1). Overall,

several organisms such as Killer whale, Pygmy chimpanzee and Sheep belonging to Amniotic origin of common ancestor showed 100% similarity to human, thus probably showing new evolutionary origin of the putative MMP7 NLS. Thus, the second hypothesis about the relatedness between the common ancestor and conservation of NLS was tested.

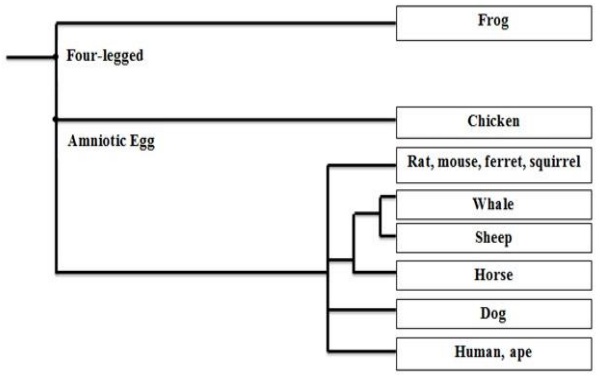

Figure 3. The cladog ram of different organisms with MMP7 NLS.

D. Interaction Maps of MMP7

Given that human MMP7 shows great differences to mouse MMP7 in both NLS and full-length protein sequences, it is interesting to find out the consequences. To determine whether MMP7 in mouse and human shows distinct function, we compared the interaction networks between human MMP7 and mouse Mmp7 using the String online service software. As shown in Fig. 4 and Fig. 5, even predominantly both human and mouse human MMP7 interact with the similar proteins such as SPP1 (Spp1), MMP9 (Mmp9), TIMP1 (Timp1), CD44 (Cd44), CDH1 (Cdh1), there are some distinct interaction proteins for human and mouse MMP7. To our surprise, human MMP7 but not mouse Mmp7 interacts with AR. As AR is one of the most important regulator for prostate cancer and we and others have shown the MMP7 expression in microenvironment of prostate cancer [6], [8]. Thus our data is consistent with the current findings that MMP7 is the potential marker for aggressive prostate cancer.

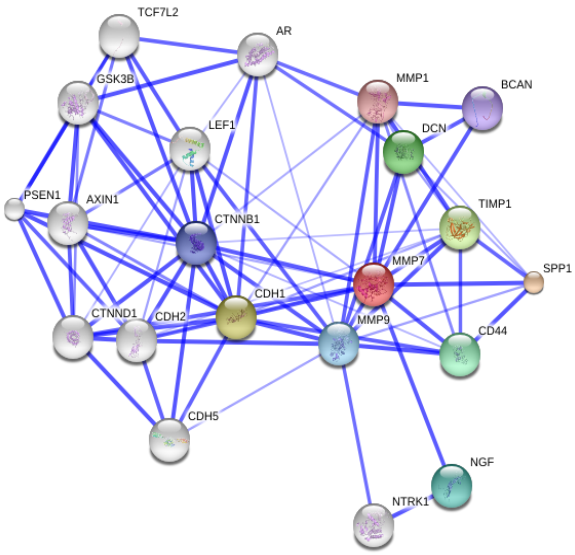

Figure 4. The interaction maps of human MMP7.

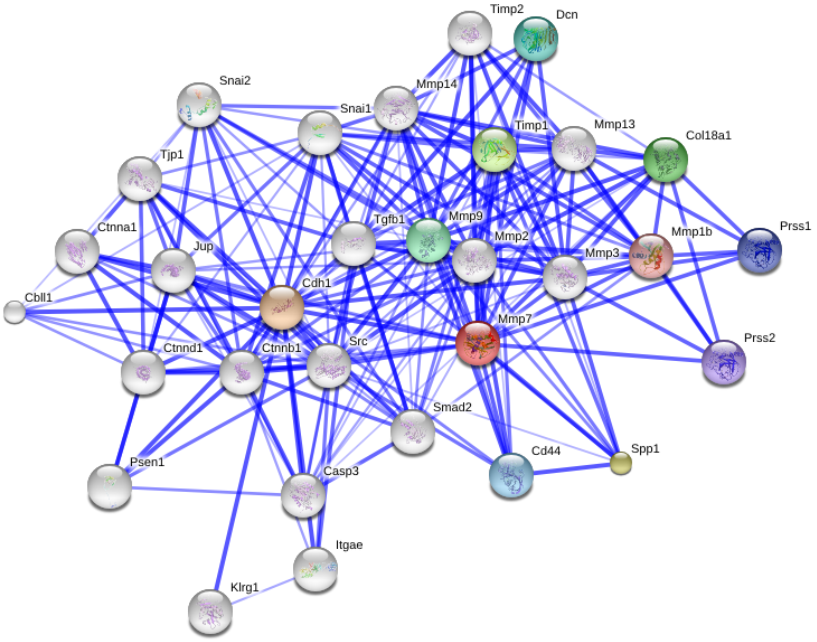

Figure 5. The interaction maps of mouse Mmp7.

We also found that compared to human MMP7, the mouse Mmp7 interacts with distinct oncogenic signaling pathways such as Src kinase, TGF beta signaling pathway (Smad, Tgfb1), Epithelial–mesenchymal transition pathway (Snail1, Snail2), and apoptosis pathway (Casp3). These may explain that mouse Mmp7 both NLS and full-length shows the sequence homologues distinct from human MMP7. Thus our data suggest that using mouse as a model system for human cancer metastasis analysis may not be ideal. Our data also suggest that evolutionary

origin of nuclear MMP7 may from a jump of natural selection.

IV. Discussion

It is known that some MMPs can lack some parts in the gene except of catalytic domain, which is significant part of the protein needed for substrate binding [3].

Therefore, putative NLS in different types of MMPs was analyzed since it is on catalytic domain of the protein (Table I). It was found that NLS was conserved in MMP7 of various species (Fig. 1).

Further, the analysis on protein BLAST database showed that Sheep, with 75% similarity to full length human MMP7, had 100% NLS similarity. While, Thirteen-lined ground squirrel with 77% similarity to human MMP7, showed only 57.14% for that in NLS. However, Pygmy chimpanzee and Killer whale with high similarity to the whole MMP7 gene, being 99% and 80% respectively, showed 100% similarity for their NLS.

Thus, high similarity to the full length MMP7 could not indicate that certain species can possess the same trend for the putative NLS.

From the before mentioned examples, we can observe that some species show higher percentage of similarity possessing ancestry from older periods, so indicating old evolutionary origin of the MMP7 nuclear localization sequence. It was hypothesized that organisms with older ancestor common to human will show less similarity of the nuclear sequence in MMP7; probably showing that NLS is new event, which leads to cancer progression. The old nuclear event could indicate the divergence and evolvement of the sequence through evolution.

V. Conclusion

By identifying the NLS similarities of proteins associated with cancer development in various species, evolutionary origin of the nuclear events in these proteins can be deduced. This could be accomplished by considering the common ancestors with human in cancer evolution. Thus, nuclear MMP7 possessing cancer causing ability was analyzed. According to NLS analysis and cladogram, it was predicted that MMP7 has new evolutionary origin. It was also found that various family members of MMPs have conservation of their nuclear localization sequence. Since different species could have higher tumor suppression ability in comparison to human, it is crucial to analyze phylogenetic origins in proteins associated with cancer. These findings have clinical relevance as the evolutional origin of nuclear MMP7 in tumor cell genotype can be identified, the prevalence of tumor progression into metastasis or therapeutic resistance can be foreseen [12]. Cancer and drug resistance are most likely driven by cascades of signaling which in most cases eventually activate nuclear events such as DNA damage and repair, regulation of transcription and cell cycle, epigenetics dynamics. Therefore, studying nuclear events of MMPs crosstalk in cancer recurrence [13], [14], cancer stemness [15], [16] and metastasis [12], [17] would provide more insight understanding of cancer evolution through multiple signaling networks [18].

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