

PERIOSTIN AND LUNG DISEASES: RECENT ADVANCES AND MOLECULAR STRUCTURE MODELING

YU TIAN, YOUFAN JIANG*, DEPENG JIANG, and YAN YANG

Department of Respiratory Medicine, Second Affiliated Hospital, Chongqing Medical University, China, 400010

Abstract - Periostin is overexpressed in many epithelial malignant cancers. Although there were some reviews on the function of periostin related to a variety of cancers, a specific review on lung cancers is not found so far. Therefore, our brief review outlines the recent advances of periostin roles in the development of lung cancers/diseases and analyzes the potential value of periostin as a therapeutic target and diagnostic signal for lung cancer. We first of all searched for previous literature on a representative set of online databases, and outlined the works on periostin expression and lung cancers. We modeled the 3D homologous structure of human periostin based on computational prediction techniques. The resulting model passed the examination of a protein quality check. The fifteen most conserved sites were identified and forty-one active binding sites were detected, which can serve as potential targets for lung cancer therapy.

Key words: Periostin, lung cancers, literature searching, homologous modeling.

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INTRODUCTION

Periostin, also known as osteoblast-specific factor 2, is a member of the fasciclin family and is a disulfide-linked cell adhesion protein (Ruan et al., 2009). Periostin is a recently identified gene that is preferentially expressed in the periosteum, indicating a potential role in bone formation and maintenance of structure (Sasaki et al., 2003). Periostin is overexpressed in many epithelial malignant cancers, including lung cancer, breast cancer, ovarian cancer and colon cancer (Hong et al., 2010).

Periostin stimulates metastatic growth by promoting cancer cell survival, invasion and angiogenesis. Therefore, periostin can be a useful marker to predict the behavior of cancer. Our brief review outlines the recent advances of periostin roles in the development of lung diseases and speculates on the usefulness of periostin as a therapeutic and diagnostic target for cancer.

Periostin is an extracellular matrix protein that has been primarily studied in the context of the heart,

where it has been shown to promote cardiac repair and remodeling. The study of Blanchard et al. (2008) focused on the role of periostin in an allergic eosinophilic inflammatory disease (eosinophilic esophagitis) known to involve extensive tissue remodeling. Periostin facilitates eosinophil tissue infiltration in allergic lung (Blanchard et al., 2008).

Periostin expression is observed in a wide range of normal adult tissues, for example, the aorta, thyroid, lung, breast, stomach, etc. Periostin overexpression is found in various types of human cancers (Kudo and Takata, 2008), e.g. breast cancer, colon cancer, lung cancer and so on. This overexpression feature makes periostin a potential diagnostic factor in detecting cancers by assessing the elevated serum periostin level in patients.

In this work we will outline recent research advances on the usage of periostin as a diagnostic indicator for lung cancer and predict the 3D homologous structure based on computational tools. Some biochemical properties (conserved amino acid positions and active binding pockets) are analyzed and predicted, correspondingly based on the mode

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Target/1-314      MIPFLPMFSLLLLLIVNPIANNNHYDKILAHSRIRGRDQGPVNCALQQILGTKKKYFSTC
Template/1-296   -----AENGALRKFYEVIMDNGGAVLDDINSLTEVTILAPSNEAWNS
                  : . . . . * . . : : : : ..

Target/1-314      KNWYKKSICGQKTTVLYECCPGYMRMEGMKGCPAVLPIDHWYGLGIVGATTTQRYSDAS
Template/1-296   SNINNVLRDRNKMQRQILNMHIKDRLNVDKIRQKNANLIAQVPTVNNNTFLYFNVRGEGS
.* :      :* : :      *:: *      :      *:.      : ...*

Target/1-314      KLREEIEGKGSFTYFAPSNEAWDNLSDIRRGLESNWNVELLNALHSHMINKRMLTKDLK
Template/1-296   DTVITVEGGGVNATVIQADVAQTNGYVHIIDHVLGVPYTTVLGKLESDPMMSDTYKMGKF
.      : ** * : . : : * * . * : . . : *. *. * : . . .

Target/1-314      NGMIIPSMYNNLGLFINHYPNGVVTVNCARIIHGNQIATNGVWHVIDRVL TQIGTSIQDF
Template/1-296   SHFNDQLNNTQRRFTYFVPRDKGWQKTELDYPSAHKKLFMADF SYHKSILERHLAISDK
. :      . : :      :      .      . : :      . . : : : : : *. *

Target/1-314      IEAEDDLSSFRAAAITSDILEALGRDGHFTLFAPTNEAFEKLPKRGVLERIMGDKVASEAL
Template/1-296   EYTMKDLVKFSQESG SVILPFRDLSIRVEEEAGRYVIIWNYKKINVYRPDVECTINGII
: . ** . * : : : . . . . . : : : : : : :

Target/1-314      MKYHILNLTQCSES
Template/1-296   HVIDYPLLEEKDVV
.      : .

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Fig. 1. Resulting alignment between human periostin (named as Target) and insect FAS1 protein (named as Template) sequences.

led 3D protein structure, which can provide insights into drug design and therapy.

Literature search

We used the keywords “periostin”, “lung”, “lung cancer” to search the following online databases: PubMed, Google Scholar, ISI Web of Knowledge, Springlink and ScienceDirect. In PubMed, 23 entries were returned. In the ISI Web of Science, 14 entries were hit. In Google Scholar, Springlink and ScienceDirect, the returned items were more than 100, most of which were not specifically related to the lung cancer.

Research advances

In summary, based on previous work on other tumors, the detailed function of periostin is multifaceted, principally including the enhancing of the

migration and invasion of cancer cells, causing cellular survival, angiogenesis and metastasis (Kudo et al., 2007). The following section is a review of previous literature related to lung cancer.

Sasaki et al. (2001) used periostin to serve as the diagnostic factor of lung cancers. Patients with periostin expression had significantly poorer survival than those without periostin expression ($p=0.0338$). Similarly, in non-small cell lung cancer, Hong et al. (2010) found that the serum level of periostin was remarkably elevated compared to normal healthy volunteers. Periostin can promote the proliferation and migration of A549 cells by inducing vimentin and N-cadherin expression and down-regulating E-cadherin expression. Ouyang et al. (2009) found similar consequences on the expression of periostin in non-small cell lung cancer cells, and they found the Akt/PKB pathway can promote cell survival. All

the above works implied that periostin is a novel molecular marker in the progression and development of lung cancers.

Takanami et al. (2008) found that periostin is secreted in 42% of total patients with non-small cell lung cancer. Furthermore, periostin expression is significantly associated with tumor size, lymph node metastasis, disease stage, etc. However, there were incongruent works. Sasaki et al. (2003) found that the serum periostin level is not elevated in lung cancer for patients with bone metastases when compared to breast cancer. The difference of periostin expression in different tissues and organs will be an interesting topic to reveal the potential role of periostin in lung cancer.

So far, we can see that work on angiogenesis has not been found on lung diseases. The vascular endothelial growth factor (VEGF) attracted much attention because it was secreted from the tumor cells, being very important for tumor growth. Further work can take the VEGF into account so as to better elucidate the mechanism of lung cancers.

Periostin expression is always high in patients with lung cancers. For example, Hong et al. (2010) found that serum periostin expression was elevated in lung cancer patients (242.84+5.33 pg/ml) compared to that of normal healthy volunteers (215.66+11.67 pg/ml) ($p < 0.05$).

It is said that periostin shares structural homology to the axon guidance protein fasciclin I (FAS1) in insects (Kudo et al., 2007). Here, we formed the homologous structure for human periostin protein based on the previously published crystal structure of FAS1 to investigate some biochemical properties of the proteins.

Molecular modeling of periostin structure

The amino acids sequence of human-originated periostin used in the modeling was downloaded from the GenBank database (www.ncbi.nlm.nih.gov). The sequence registration number is access number: Q15063.2, which has 836aa. The template of the 3D protein structure of FAS1 was obtained from the RCSB database (www.pdb.org). The template structure is

1O70, the FAS1 domain pair from the insect cell adhesion molecule fasciclin I (Clout et al., 2003).

Sequence alignment for periostin was used to drive a 3D model by Modeller 9v7 (Eswar et al., 2006). Five rough 3D models based on multiple templates were constructed with a default of parameters that proposed loop conformations. The best model was chosen and the quality of the structure was studied. Energy minimization calculations for the 3D model of M1 protein were performed using a GROMOS 96 4B1 parameter set energy minimization package of SWISS PDB viewer (<http://spdbv.vital-it.ch/>).

The quality and validation of the final candidate model was analyzed by the SAVS info server (http://nihserver.mbi.ucla.edu/SAVES_3/saves.php). The stereochemical quality of the models was assessed by PROCHECK (Laskowski et al., 1993), which analyzes aspects of main-chain and side-chain geometry. Ramachandran analysis allows identification of the number of residues with non-ideal torsion angles. Root mean-square deviation (RMSD) allowed the comparison of the models to their corresponding templates.

The conserved regions were identified and mapped onto the protein structures using the web-based ConSurf server (<http://consurf.tau.ac.il/>), providing the protein structure file as input. The degree of conservation is subdivided into nine grades, with grade 1 being the least and grade 9 being most conserved (Ashkenazy et al., 2010).

Active binding sites for the model were predicted by the tool of the computed atlas of surface topography of protein (CASTp) (<http://sts.bioengr.uic.edu/castp/calculation.php>). CASTp is an online server which predicts the binding sites and active sites of proteins and DNAs and is often associated with structural pockets and cavities (Liang et al. 1998).

The resulting sequence alignment and the generated homologous periostin 3D structure are shown in Figs. 1 and 2, respectively. Based on the conservation scores, fifteen positions were found as the most conserved residues, including ALA273, THR133, ALA136, PRO137, ALA141, HIS168,

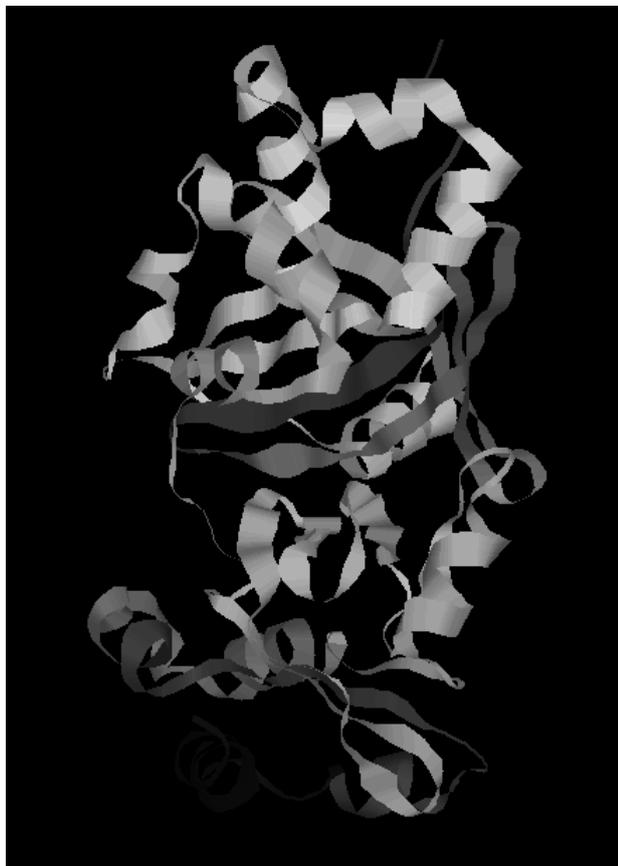


Fig. 2. Resulting 3D model generated from the MODELLER program.

SER187, ASN220, GLY221, HIS224, ILE226, THR270, PRO274, ASN276, ALA278, and HIS304.

A total of 41 active sites were identified by the CASTp server; however, the low-ranked active sites are very small and share several positions with the top sites. We only show the top two sites here (Fig. 3). The full list of active sites is available upon request from the authors.

The first binding site has an area of 1831.3 Å² and volume of 2711.9 Å³. The 66 amino acid positions included are: ALA110, ALA164, ALA243, ALA296, ALA299, ARG289, ASN156, ASN171, ASN190, ASN276, ASN62, ASP245, ASP246, ASP293, ASP99, CYS79, GLN114, GLN71, GLU125, GLU127, GLU154, GLU244, GLU277, GLU288, GLY103, GLY106, GLY109, GLY128, GLY130, GLY292, HIS168, HIS198, ILE107, ILE126, ILE170, ILE241, ILE290, ILE98, LEU300, LEU76, LYS129, LYS294, LYS302, LYS61, LYS65, LYS66, LYS72, MET169, MET291, PHE132, PHE240, PHE272, SER155, SER167, THR104, THR111, THR112, THR113, THR275, TRP63, TYR83, VAL101, VAL108, VAL157, VAL295, and VAL75. The second active site has an area of 759.8 Å² with a volume of 896.3 Å³. Thirty three amino acid positions comprise the site including ALA30, ARG33, ASN42, ASN62, ASP146, ASP26, ASP38, CYS80, GLN39, GLN48, GLY36, GLY51, HIS24, HIS31, ILE28, ILE34, LEU153, LEU50, LEU76, LYS27, LYS53, PRO81, SER147, SER32, SER58, SER67, THR52, THR59, THR73, TRP142, TRP63, TYR64, and TYR77.

Chain 0

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19-  I N A N N H Y D K I  L A H S R I R G R D  Q G P N V C A L Q Q  I L G T K K K Y F S  T C K N W Y K K S I
69-  C G Q K T T V L Y E  C C P G Y M R M E G  M K G C P A V L P I  D H V Y G T L G I V  G A T T T Q R Y S D
119- A S K L R E E I E G  K G S F T Y F A P S  N E A W D N L D S D  I R R G L E S N V N  V E L L N A L H S H
169- M I N K R M L T K D  L K N G M I I P S M  Y N N L G L F I N H  Y P N G V V T V N C  A R I I H G N Q I A
219- T N G V V H V I D R  V L T Q I G T S I Q  D F I E A E D D L S  S F R A A A I T S D  I L E A L G R D G H
269- F T L F A P T N E A  F E K L P R G V L E  R I M G D K V A S E  A L M K Y H I L N T  L Q C S E S

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Fig. 3. Two top active binding sites from the predicted 3D homologous structure with different grey colors.

CONCLUSIONS

Based on published literature, we outline the potential objectives for studying periostin in lung cancer, such as migration of invasion, angiogenesis and so on. All these aspects have not been considered in previous works, but have been discussed in other cancers.

Periostin can serve as a target in chemotherapy in certain cases of human cancers. Thus, in the second part of our study we also made an attempt to model the 3D molecular structure of periostin, which does not have an experimental crystal structure so far. The model settles for protein quality checks and has 41 active binding sites in total. These active sites could serve as potential drug targets for lung cancer therapy.

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