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生物大分子结构与功能课程论文

题	目	Polyubiquitin chains
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Polyubiquitin Chains

Abstract . Ubquitin is a small protein which belongs to the family of structural conserved proteins . It governs many biological processes in eukaryotic cells through covalent conjugation to other proteins include Poly-Ub or Ub itself . Many paper has published to explain the function of polyubiquitin chains in nearly a decade , such as the Lys48-linked polyubiquitin chain and the Lys63-linked polyubiquitin chain . But other polyubiquitin chains have few research results . This review summerizes the latest years advances about the founction of polyubiquitin chains and discusses the potential mechanism of chain linkage, which determines the various function of polyubiquitin chains.

Keywords: Ubiquitin , polyubiquitin chains , proteasome , conjugation .

Introduction

Ubiquitin(Ub) is the consered protein consisting of 76 amino acids in eukaryotes [1] . It can exeute different function through covalent attachment to various target proteins in the form of a Mono-Ub or polymeric Ub chains . Ub plays an vital role as a sign of protein degredation to up-regulate or down-regulate cell cycle progression in celluar processes [2 , 3] . Maybe the perfect understanding of the function of Ub is that bonding to specific proteins for degradation by 26S proteasomes [4] . The Ub-proteasome Systeam(UPS)（Figure.1） is the principal meachanism for the turnover of short-lived proteins , which requires three ubiquitin enzymes , named E1 , E2 and E3 [5] . Firstly , Ub is activated by Ub-activating enzyme(E1) with the help of ATP , forming a thiol ester with the carboxyl group of Ub ; Secondly , Ub is conveyed to a Lys residuce of the Ub-conjugating enzyme(E2) ; At last , Ub is conveyed to a Lys residue of target protein which depends on Ub-ligasing enzyme(E3) [6] . This conjugation cascade is a complex but accurate precess , it usually includes a single E1 , a special E2s and several E3s , all of them are important to UPS .

Seven lysine residuces and an N-terminal methionine (M1) are in Ub , they are Lys6 , Lys11 , Lys27 , Lys33 , Lys48 , Lys63 and G76. The polyubiquitin chain can linked through all of them , so it can form many kinds of Poly-Ub chains , of which have different functionam readouts [7] . Substrates attached to a single Ub are difficult to recongnise by 26S proteam to degradate in vitro , but a chain including at least four Ubs is more efficient [8] .

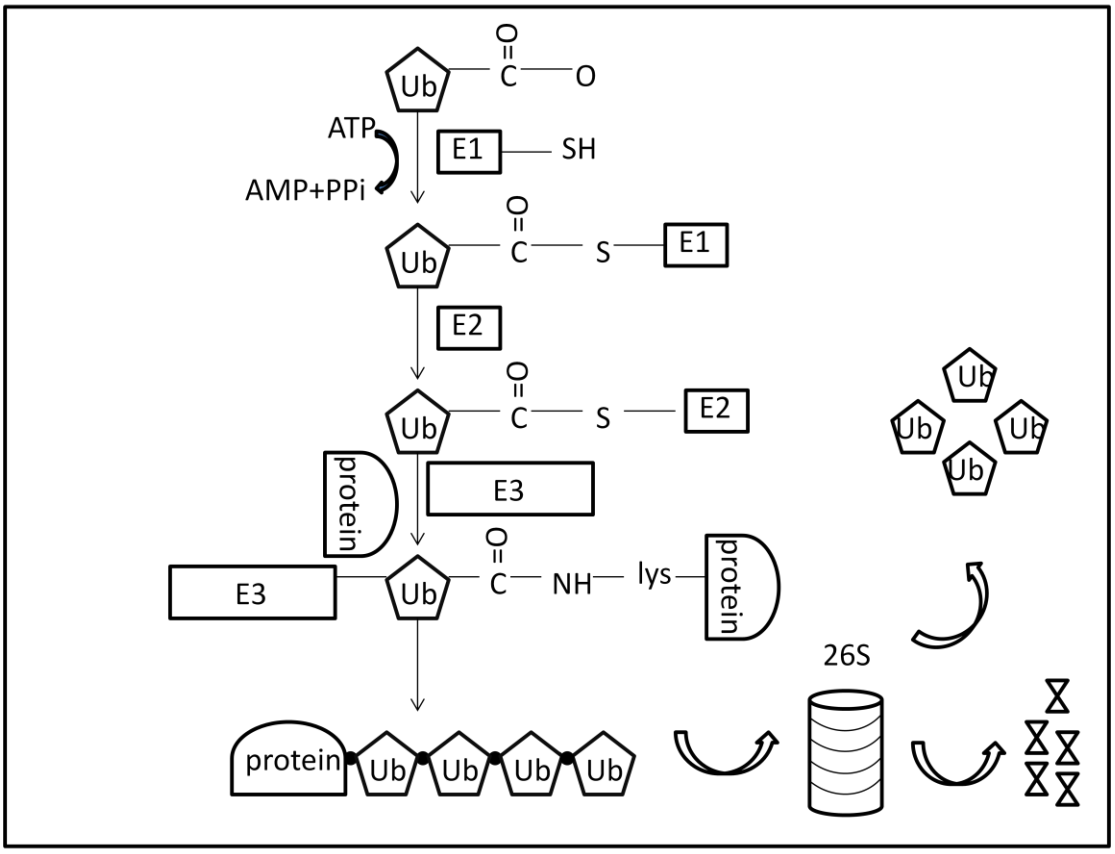

Figure . 1

Figure .1 The Ub-proteasome Systeam(UPS) . The substrate protein is conjugated to the polyubiquitin chain through the catalysis of E1 , E2 and E3 ubiquitin enzymes . The protein which is marked by polyubiquitin chain can be reconginated by 26S proteasome and then degradation .

The K48-linked polyubiquitin chains

What is known to us is that the function of K48-linked polyubiquitin chain is to target the substrates for degredation by 26s proteasome [9] . But it also has some nonproteolytic functions , for expmble , it regulates the activity of the transcription factor Met4 *S.cerevisiae* through conjugating with Met4 [10] . What surprised us is that Met4 is still long-lived protein although conjugating with the K48-linked polyubiquitin chain [11] . Later study proves that Met4 has a ubiquitin-binging domain to conjugate with ubiquitin chains to limit the length of the chain which is required for proteasome recognition [12] . The K48-linked polyubiquitin chain also regulates the activity of a ubiquitin-selective , chaperone named p97 [9] . Although it is essential to animals , it has a indispensable function in plant [13] .

53BP1 , the tumor suppressor , which is a key protein needed to regulate DNA repair . However , it is reported that the K48-linked polyubiquitin chain palys an crucial role in DNA repair , it controls the aggregation of the 53BP1 to the sides of DNA damage [14] . Another report reveals that with the help of ubiquitin-selective segregase VCP/p97 , which is aggregated to DNA breaks in an RNF8-depended

model requiring the K48-linked polyubiquitin chain , the K48-linked polyubiquitin chain can promote the chromatin extraction of protein [15] .

The K63-linked polyubiquitin chains

K63-linked polyubiquitin chains are the other kind of ubquitin chain which has been detected a lot by people . It is main function containing protein synthesis , chromosome regulation , kinase activation and DNA repair[16]. But it has different functions with K48-linked polyubiquitin chains which can serve as degradation purpose . Do K63-linked polyubiquitin chains work in proteolysis , some researches have revealed a little information [17,18] , but other survery suggests that it makes substrates degradated fate through a proteasome-independent mechanism named autophagy .

Autophagy is a celluar process involved in the so-called autophagasome which degrades the sequested contents [19] . Tt not only makes cells to fight with stress conditions , but also can it delete misfolded protein aggregates and damaged organelles which are too big to be degraded through the proteasome [9] . P63 is a member which belongs to Ubl/UBA protein family , it can recongnized some misfolded proteins attaching with K63-linked polyubiquitin chains [20] , but its mechanism is still not clear . The function of K63-linked polyubiquitination is also involved in a list of celluar events [9] .

Other types of polyubiquitin chains

K48- and K63-linked polyubiquitin chains have been detected a lot by researchers by now , they are involved in many mechanism in both organism and plants [21] . But other types of linked chains begain to explore in recent years . Compared to K48- and K63-linked polyubiquitin chains , the K6- , K11- , K29- , K33-linked polyubiquitin chain have litter research .

Reasearches about K29-linked polyubiquitin chains involved in the degradation of DELLA proteins , which is a important protein in the plant . It reveals that K29-linked chains can target the DELLA protein to 26s proteasome for degradation [22] . It means that K29-linked poly- ubiquitin chain can play a similar to the K48-linked chains .Otherwise , the K29-linked polyubiquitin chain exists in cell in the form of mixed or branched chains which contains other linkages [23] . K6- , K11- , K27- and K33-linked polyubiquitin chain are tend to systhesis in eukaryotes such as *S.cerevisiae* [12]. Although the function of these chains have little information , they play non-ignorable roles in their aspects .

AIP4 , one of the Hect domain E3 ligase , makes K29-linked polyubiquitin chains on Notch signaling modulator DTX to induce it for lysosomal degradation [24] . BRCAI E3 complex , can assemble K6-linked and K29-linked ubiquitin chains , it also regulates the stability of the E3 enzyme [25] . Modification of AMPK-related kinases are related with K29-linked ubiquitin chains in cell , which regulates the activity of kinases [26] . Modification of AMPK-related kinses related with K29-linked and K33-linked polyubiquitin chains in the cell , which regulates the activity of kinases [27] . The M1-linked polyubiquitin chain is conjugating through the methionine

residue , which is located at the N-terminas [28] . It is a liner chain that plays an significant role in mammaliam signaling pathways , including tumor necrosis factor (TNF) [29] and NF-KB [30] .

However , the examples above all can indicted that every kinds of polyubiquitin chain has its unique function , they are all indispensability in life cycle . A lot of information about polyubiquitin chains wait us to be founded , these reports also make us pay attention to the further investigation (Figure.2).

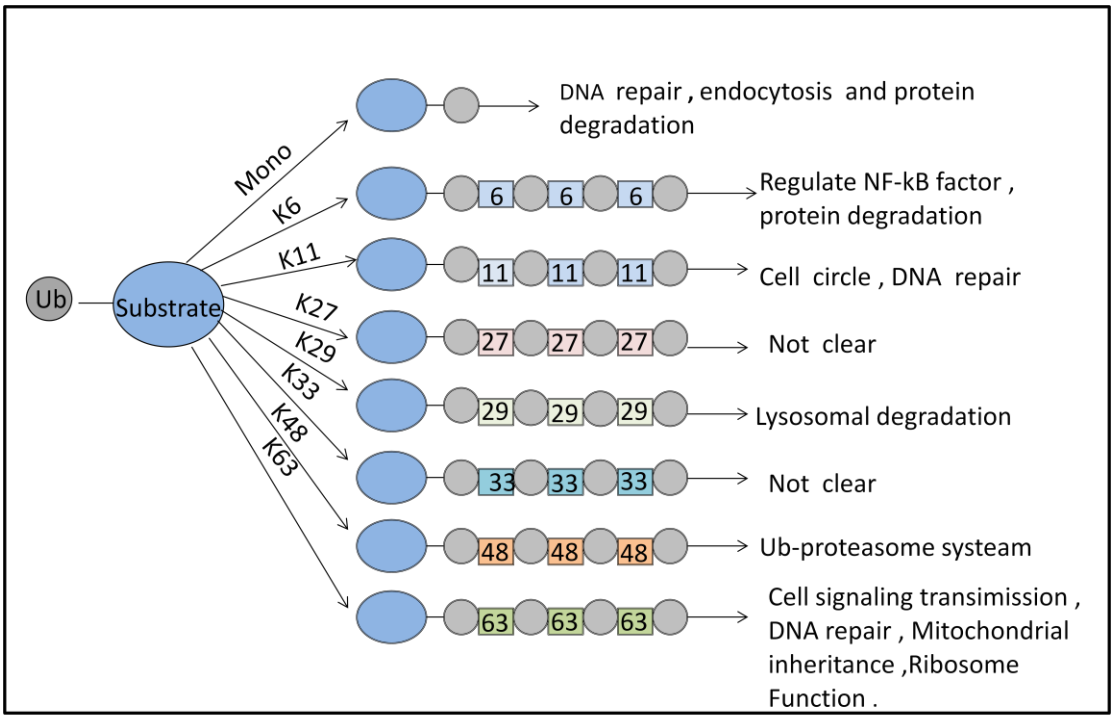

Figure . 2

Figure . 2 The function of different kinds of polyubiquitin chain . Different color represents different polyubiquitin chains . M1-ubiquitin chains and K63-linked polyubiquitin chains are main for DNA repair ; K6-linked polyubiquitin chains and K48-linked polyubiquitin chains are for protein degradation ; K27-linked polyubiquitin chains and K33-linked polyubiquitin chains is not clear ; K29-linked polyubiquitin chains are for lysosomal degradation . K11-linked polyubiquitin chains is for cell circle . They all play an important role .

Mechanism of linkage selectivity

As we know , Ub contains seven lysine residues , so they can form many kinds of polyubiquitin chains , different chain linkages have their special functions in biological processes . However , what's the mechsnism of linkage selectivity ? Why it can form the special linkage chains ?

Tow ubiquitin molecules , one called donor Ub and the other called acceptor Ub , are needed to form a polyubiquitin chain . The donor Ub is linked to ubiquitin conjugating enzyme(E2) or the special Hect domain E3 enzyme at first and then it

ligates to a lysine residue of the acceptor Ub [31] .

Some researches have reported that the formation of a special ubiquitin linkage are determinate by several amino acid sites near the linkage site . KIAA10 , a Hect domain E3 enzyme , can catalyze K29- and K48-linked polyubiquitin chains [32] . Wang and her colleagues mutanted the amino acid sites of ubiquitin one by one . They found that E18 , E16 and D21 amino acid sites that affecting the sythnesis of K29-linked polyubiquitin chains , if those sites mutanted , it cannot sythnesis the K29-linked polyubiquitin chain .Tt is the same with K48-linked polyubiquitin chains , whose linkage selectivity is determinated by F45 and Y59 amino acid sites [33] . Ubc9-RanGAP1 interaction is also similar with them , their linkage sit is Lys526 ,which ia related with Tyr87 , Cys93 , Asp127 amino acid sits [34] . As these related sits are all near to the linkage sites , we call it direct mechanism . Another example is about the K63-linked polyubiquitin chain . Previous researches have been reported that the K63-linked polyubiquitin chain , which needs Mms2 • Ubc13 complex (UEV/E2) to sythnesis [35] . Form its crystal structure , we can conclude that the acceptor Ub-I44 and Mms2-I57 are important to the orientation of acceptor Ub , so its K63 sit are right to the G76 of dorner Ub [36 , 37 , 38] . In constant , those amino acid sits are distant from the linkage site , so we call it indirect mechnasim .

At present , the mechanism of other polyubquitin chains linkage selectivity are not clear enough , but I believe those results will arouse more people to investigating the further secret of polyubiquitin chains .

Perspective

Recent year , many researches have been done to study the basic biology and biochemisty of the ubiquitin protein family , which still have many new things to be discovered . Our knowlwdge of selectivity machinery and conjugating machinery about ubiquitin-related proteins is very scare , but one point is clear that the srlectivity machinery of linkage is related with the other amino acide residuces and the biochemical conjugating machinery is highly conserved .

Ubquitin is an important peptide modifier which is formed by differing the selection of E1 , E2 and E3 enzyme , and thus lead to the polyubiquitin chains of different linkage . Different linkages have different function . As many structure of polyubiquitin chains and related enzymes have been showed , if we want to know more mechanism of polyubiquitin chains , more structure work should be done in the future . At the same time , the intensity of endeavor focused on the pathway has also played an important role in the mechanism that we still understand . About uniquitin and ubquitin chains , we have many work required to do , previous work enhance our confidence to do more work and have original discover .

Reference

1. Marx, J. (2002) Ubiquitin lives up to its name . *Science* 297 , 1792–1794 .
2. Pickart CM, Eddins MJ (2004) Ubiquitin : structures , function , mechanisms . Biochem Biophys Acta 1695: 55–72 Hershko , A. , and Ciechanover , A. (1998) *Annu . Rev . Biochem.*

67, 425–479 .
3. Pickart CM (2004) Back to the future with ubiquitin . Cell 116: 181-190 .
4. Hershko , A. , and Ciechanover , A. (1998) . *Annu . Rev . Biochem.* **67,** 425–479 .
5. Pickart , C.M. (2000) Ubiquitin in chains. *Trends Biochem . Sc i.* 25 , 544–548 .
6. Schulman , B. A. , and Harper , J. W. (2009) . Ubiquitin-like protein activation by E1enzymes : the apex for downstream signalling pathways . *Nat. Rev. Mol. Cell. Biol.*10, 319–331. doi: 10.1038/nrm2673 .
7. Spratt , D. E. , Wu , K. , Kovacev, J., Pan, Z. Q., and Shaw , G. S. (2012) . Selectiverecruitment of an E2～ ubiquitin complex by an E3 ubiquitin ligase . *J. Biol.Chem .* 287, 17374–17385 . doi: 10.1074/jbc.M112.353748 .
8. Finley , D., Sadis , S., Monia , B. P. , Boucher , P., Ecker , D. J., Crooke , S. T. , and Chau , V. (1994) *Mol. Cell. Biol.* **14,** 5501–5509 .
9. Thrower , J.S., Hoffman , L. , Rechsteiner , M. and Pickart , C.M. (2000) . Recognition of the polyubiquitin proteolytic signal . EMBO J. 19, 94–102 .
10. W. Li and Y. Ye（2008）Polyubiquitin chains: functions, structures, and mechanisms . Cell. Mol. Life Sci. 65 (2008) 2397 – 2406 .
11. Kaiser, P. , Flick, K. , Wittenberg, C. and Reed , S.I. (2000) . Regulation of transcription by ubiquitination without proteolysis: Cdc34/SCF (Met30)-mediated inactivation of the transcription factor Met4. Cell 102, 303–314 .
12. Flick , K. , Raasi , S., Zhang, H. , Yen , J.L. and Kaiser, P. (2006) . A ubiquitin-interacting motif protects polyubiquitinated Met4 from degradation by the 26S proteasome. Nat. Cell Biol. 8,509–515 .
13. *Charlotte K. Walsh and Ari Sadanandom* (2014) . Ubiquitin chain topology in plant cell signaling : a new facet to an evergreen story . Plant Sci. 2014 Apr 1;5:122. doi: 10.3389/fpls.2014.00122 . eCollection 2014 .
14. Mallette FA , Richard S (2012) . K48-linked ubiquitination and protein degradation regulate 53BP1 recruitment at DNA damage sites. Cell Res. 2012 Aug;22(8) : 1221-3. doi: 10.1038/cr.2012.58. Epub 2012 Apr 10.
15. Meerang M, Ritz D, Paliwal S, et al (2011) . The ubiquitin-selective segregase VCP /p97 orchestrate the response to DNA double-strand breaks. Nat Cell Biol. 2011;13: 1376–1382.
16. Jacobsen , A. D. , Zhang, N. Y., Xu, P., Han, K. J., Noone, S., Peng, J., et al. (2009) . The lysine 48 and lysine 63 ubiquitin conjugates are processed differently by the 26S proteasome. *J. Biol. Chem.* 284, 35485–35494. doi: 10.1074/jbc.M109.052928 .
17. Kim , H.T. , Kim , K.P. , Lledias , F. , Kisselev, A.F. , Scaglione,K.M., Skowyra, D., Gygi, S.P. and Goldberg, A.L. (2007) Certain pairs of ubiquitin-conjugating enzymes (E2 s) and ubiquitin-protein ligases (E3 s) synthesize nondegradable forked ubiquitin chains containing all possible isopeptide linkages. J. Biol. Chem. 282, 17375–17386 .
18. Babu, J.R., Geetha, T. and Wooten, M.W. (2005) . Sequestosome 1/p62 shuttles polyubiquitinated tau for proteasomal degradation. J. Neurochem. 94, 192–203.
19. Reggiori, F. and Klionsky, D.J. (2005) . Autophagosomes: biogenesis from scratch? Curr. Opin. Cell Biol. 17, 415–422.
20. Seibenhener, M.L., Babu, J.R., Geetha, T., Wong, H.C. , Krishna , N.R. and Wooten , M.W. (2004) Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in

ubiquitin proteasome degradation. Mol. Cell. Biol. 24, 8055–8068 .

21. Li, W., and Schmidt, W. (2010) . A lysine-63-linked ubiquitin chain-formingconjugase, UBC13 , promotes the developmental responses to iron deficiencyin Arabidopsis roots. *Plant J*. 62, 330–343. doi: 10.1111/j.1365-313X.2010.04150.x
22. Wang, F., Zhu, D., Huang, X., Li, S., Gong, Y., Yao, Q., et al. (2009) . Biochemical insights on degradation of *Arabidopsis* DELLA proteins gained form a cell-free assay system. *Plant Cell* 21, 2378–2390. doi: 10.1105/tpc. 108.065433 .
23. Yosua Adi Kristariyanto,1 Syed Arif Abdul Rehman,1 David G. Campbell,1 Nicholas A. Morrice,1,2 Clare Johnson,1Rachel Toth,1 and Yogesh Kulathu (2015) . K29-Sel ective Ubiquitin Binding Domain Reveals Structural Basis of Specificity and Hetero typic Nature of K29 Polyubiquitin. Mol Cell . 2015 Apr 2;58(1):83-94. doi: 10.101 6/j.molcel.2015.01.041 . Epub 2015 Mar 5 .
24. Wang, F., Zhu, D., Huang, X., Li, S., Gong, Y., Yao, Q., et al. (2009) . Biochemical in sights on degradation of *Arabidopsis* DELLA proteins gained form a cell-free assay system. *Plant Cell* 21, 2378–2390. doi: 10.1105/tpc. 108.065433 .
25. Al-Hakim, A.K., Zagorska, A., Chapman, L., Deak, M.,Peggie, M. and Alessi, D.R. (2008) . Control of AMPK-related kinases by USP9X and atypical Lys29/Lys33-linked polyubiquitin chains. Biochem. J.
26. Chastagner, P., Israel, A. and Brou, C. (2006) . Itch/AIP4 mediates Deltex degradation through the formation of K29-linked polyubiquitin chains. EMBO Rep. 7, 1147–1153.
27. Nishikawa, H., Ooka, S., Sato, K., Arima, K., Okamoto, J.,Klevit, R.E., Fukuda, M. and Ohta, T. (2004) . Mass spectrometric and mutational analyses reveal Lys-6-linked polyubiquitin chains catalyzed by BRCA1-BARD1 ubiquitin ligase. J. Biol. Chem. 279, 3916–3924.
28. Tokunaga, F., Sakata, S., Saeki, Y., Satomi, Y., Kirisako, T., Kamei, K., et al. (2009) . Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat. Cell. Biol.* 11, 123–132. doi: 10.1038/ncb1821
29. Emmerich, C. H., Schmukle, A. C., and Walczak, H. (2011) . The emerging role of linear ubiquitination in cell signalling. *Sci. Signal.* 4, re5. doi: 10.1126/scisignal.2002187
30. Haas, T. L., Emmerich, C. H., Gerlach, B., Schmukle, A. C., Cordier, S. M., Rieser, E., et al. (2009). Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signaling complex and is required for TNF-mediated gene induction. *Mol. Cell* 36, 831–844. doi: 10.1016/j.molcel.2009.10.013
31. Aillet, F., Lopitz-Otsoa, F., Hjerpe, R., Torres-Ramos, M., Lang, V., and Rodríguez,M. S. (2012). Isolation of ubiquitylated proteins using tandem ubiquitinbinding entities. *Methods Mol. Biol*. 832, 173–183. doi: 10.1007/978-1-61779-474-2_12
32. Tokunaga, F., Sakata, S., Saeki, Y., Satomi, Y., Kirisako, T., Kamei, K., et al. (2009) . Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat. Cell. Biol.* 11, 123–132. doi: 10.1038/ncb1821
33. Mastrandrea LD, You J, Niles EG, Pickart CM (1999) . E2/E3-mediated assembly of lysine 29-linked polyubiquitin chains. J Biol Chem 274: 27299–27306
34. Min Wang , Dongmei Cheng , Junmin Peng and Cecile M Pickart (2006) . Molecular determinants of polyubiquitin linkage selection by an HECT ubiquitin ligase .The EMBO Journal (2006) 25, 1710–1719 .
35. Bernier-VillamorV, SampsonDA, MatunisMJ, CD.(2002) .Structural basis for E2-mediated

SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1. Cell. 2002 Feb 8;108(3):345-56.

36. Ulrich HD (2004) . How to activate a damage-tolerant polymerase: consequences of PCNA modifications by ubiquitin and SUMO.Cell Cycle 3: 15–18 .
37. Bernier-Villamor V , Sampson DA , Matunis MJ , Lima CD.（2002）. Structural ba sis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conj ugating enzyme Ubc9 and RanGAP1.
38. Michael J Eddins1, Candice M Carlile2, Kamila M Gomez1,3, Cecile M Pickart2,4 & Cynthia Wolberger1（2006）. Mms2–Ubc13 covalently bound to ubiquitin reveals the structural basis of linkage-specific polyubiquitin chain formation. Nat Struct Mol Biol. 2006 Oct;13(10):915-20. Epub 2006 Sep 17.

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Molecular mechanism of diabetic nephropathy

Abstract

Diabetes is the major cause of chronic kidney disease which in turn may lead to end-stage renal disease(ESRD) ending up in dialysis. Hemodynamic and structural changes following diabetes are working together in the process of development of diabetic nephropathy (DN). Hyperglycemia-induced metabolic and hemodynamic pathways are proven to be the mediators of kidney disease. Hyperglycemia causes the formation of Amadori products, which are the altered proteins and advanced glycation end products (AGE) are the molecular players in the phases of DN. According to recent studies, activation of electron transport chain induced by hyperglycemia can result in an increase in the reactive oxygen species (ROS) formation, which is thought to be the initiating event in the development of complications in diabetes. Hemodynamic changes, hypertrophy, extracellular matrix accumulation, growth factor/cytokine induction, ROS formation, podocyte damage, proteinuria, and interstitial inflammation are the steps in the advancement of DN. High glucose, AGEs, and ROS act in unison to induce growth factors and cytokines through signal transduction pathways.

Key words: Diabetic nephropathy, signal pathways, ROS, AGEs

Introduction

Diabetes mellitus is now one of the leading causes of renal failure in the world. About 40% of all type 1 diabetic patients develop diabetic nephropathy. Since there is a considerable ethnic variability of the incidence of diabetic nephropathy and because not all but only about one third of type 1 diabetics develop diabetic nephropathy a genetic contribution is very likely. Although relatively less type 2 diabetic patients develop nephropathy promoting to end stage renal disease, this group of patients is getting more important because of the rapidly growing number of type 2 diabetic patients. Therefore, the knowledge of the molecular mechanism of the pathogenesis of the diabetic nephropathy may help to develop new therapeutic concepts and to find diagnostic markers for early detection of diabetic nephropathy.

1. The role of signal pathway in diabetic nephropathy

Glomerular hemodynamic changes occurs very early in DN, which include hyperfiltration and hyperperfusion injuries. There is a decrease in both afferent and efferent arteriolar resistance which is more on the afferent side leading to increased glomerular capillary pressure that enhances trans-capillary hydraulic pressure gradient as well as an increase in glomerular plasma flow. Hyperperfusion and hyperfiltration are also said to be due to factors such as prostanoids, nitricoxide, atrial natriuretic factor, growth hormone, glucagon, insulin and angiotensin-II

(ANG-II). The changes leading to glomerulosclerosis are elevated intraglomerular pressure, increase in mesangial cell matrix production and thickening of glomerular basement membrane. Hyperglycemia stimulate the synthesis of ANG-II, which exert hemodynamic, trophic, inflammatory and profibrinogenic effects on renal cells. The factors that mediate hyperfiltration injury include vascular endothelial growth factors (VEGF) and cytokines such as transforming growth factor-beta (TGF-β). The key role in diabetic vascular derangement can be attributed to TGF-β . A basic mechanism underlying diabetic nephropathy appears to be the high glucose(HG)-induced overexpression of transforming growth factor-β (TGF-β) and the accumulation of extracellular matrix(ECM) molecules, such as collagen IV and fibronectin. Furthermore, the other mechanism is the increase NO production by the up-regulation of endothelial NO synthase (eNOS) mRNA expression and by enhancing arginine resynthesis.

Alteration of glomerular hemodynamics due to shear stress and mechanical strain, induce the autocrine and/or paracrine release of cytokines and growth factors. Hemodynamic stress causes structural changes of DN by the local activation of cytokines and growth factors. Increase in reabsorption of sodium chloride in proximal tubules or loops of Henle leads to an increase in the glomerular filtration rate by an intact macula-densa mechanism and hypertrophy of tubules that mediate

stimulated sodium chloride reabsorption could be pivotal in this process, linking again structural changes with hemodynamic adaptation in DN.

a. The association of TGF-β with diabetic nephropathy

Transforming growth factor-β is a profibrotic growth factor causing the expansion of mesangial matrix and renal hypertrophy in DN.

High levels of TGF-B have been measured in the glomeruli of strptozotocin diabetic rats. It was reported that neutralizing TGF-β antibody prevented diabetic renal atrophy, mesangial matrix expansion, and the development of renal failure in type 2 db/db mice. Connective tissue growth factor and heat shock proteins, which are encoded by TGF-β , have fibrogenic effects on the kidneys of patients with diabetes. However, the profibrogenic actions of TGF-β 1 are countered by the decreased expression of renal bone morphogenic protein. Mechanical stretch induces both gene and protein expression of TGF-β 1. Stretch, via the intra cellular signaling molecule protein kinase C, causes early activation of p38 mitogen-activated protein kinase, which induces TGF-β 1 and fibronectin production. The TGF-β 1 contributes to the cellular hypertrophy and increased synthesis of collagen, which inturn leads to DN. The platelet derived growth factor-beta(PDGF-β) cause histological alterations in the glomerulus. Hyperglycemia up-regulates PDGF-β growth factor and its receptor in the mesangial cells leading to enhanced TGF-β expression.

TGF-β has been acknowledged to mediate glomerulosclerosis and interstitial fibrosis in renal diseases. The expression of TGF-β 1 is up-regulated in renal cells incubated in high glucose conditions and in the kidney of animal models of diabetes, including the streptozotocin (STZ)-diabetic rat or mouse and the db−/db− mouse. The TGF-β signaling pathway is predominantly transduced by a family of transcription factors, the Smad proteins. After binding the TGF-β , the type II receptor activates the type I receptor kinase, which next phosphorylates Smad2 and Smad3. Then Smad2 and Smad3 combine with Smad4 into a complex, which is translocated into the nucleus and then up-regulates ECM genes, including FN and type I Collagen. So Smad3 exerts powerful effects on ECM accumulation in mesangial cells exposed to high glucose or TGF-β 1. It is reported that TGF-β action can be suppressed by several factors such as insulin, insulin-like growth factor-1(IGF-1), fibroblast growth factor, interleukin-6 and interleukin-4, and even over expression of insulin receptor substrate1 via activation of PI3K/Akt pathway and TGF-β - stimulated Smad pathway mediated transcriptional responses is also inhibited by insulin and IGF-1. It is therefore possible that cross-talk between Smad pathway and PI3K/Akt pathway may affect ECM production in mesangial cells.

Just like TNFα and interleukins, the chemokine families involve in the migration of intravascular white blood cells to inflammation regions

during an immune response. Chemokines can be divided into four types, including CC, CXC, CX3C and C. Chemokine(C–C motif) ligand2, also known as MCP-1 is an extensively studied chemokine in chronic kidney disease. Animal studies have found that MCP-1 blockade can ameliorate chronic kidney diseases, such as lupus-like kidney disease and diabetic nephropathy. Stromal cell-derived factor- 1 (SDF-1)is classified into two subfamilies, including stromal cell- derivedfactors1-α and1-β , which are small cytokines that belong to the inter crinefamily. SDF-1regulates numerous homeostatic, developmental, and pathological processes through its receptors CXCR4 by inducing several signaling transduction pathways, including activation of PI3K/Akt pathway. SDF-1 plays an important role in tissue repairing by a mechanism of regulating migration of cells. Some studies found that SDF-1α promoted the pulmonary fibrosis. In addition, emerging evidences demonstrated that SDF-1α had a role of cardiac protection in myocardial infarction and reduced infarct size and fibrosis. Recently, studies found that functional blockade of SDF-1α significantly improved diabetic nephropathy.

TGF-β -induced apoptosis is critical in development and tissue homeostasis, and is also important for its tumor suppressor activity. Although the mechanisms of TGF-β -induced apoptosis vary among different cell types, the intracellular signaling protein Smad3 functions as a key mediator in TGF-β -induced apoptosis. For example, TGF-β

induces apoptosis in heptocytes through Smad3-dependent cleavage of BAD, or through Smad2, Smad3 and Smad4-mediated expression of DAP kinase. TGF-β induces apoptosis in hepatocytes and B-lymphocytes through Smad3-dependent transcription of the MAPK phosphatase MKP2, which enhances the proapoptotic effect of the Bcl-2 family member Bim. Our group has previously reported that Smad3 plays an essential role in TGF-β -induced apoptosis in hepatocytes and lung epithelial cells, and Akt interacts with Smad3 to inhibit Smad3-mediated transcription and apoptosis.

Parathyroid hormone-related protein (PTHrP) is a polyhormone secretory protein that plays a critical role in a number of biological processes by acting via paracrine, autocrine and intracrine pathways. PTHrP was identified as a tumor-derived humoral factor that causes humoral hypercalcemia of malignancy. There are three isoforms of human PTHrP peptides ranging in length from 139 to 173 residues, all of which are subjected to extensive post-translational processing to generate multiple secretory isoforms of mature peptides representing the N-terminal, mid-region, and C-terminal portions of the protein. The N-terminal 1 – 13 amino acids of PTHrP are highly homologous with parathyroid hormone (PTH). The mid-region of PTHrP contains a bipartite nuclear/nucleolar localization signal. PTH and PTHrP act through a common receptor, the type I PTH receptor (PTHR1), which is a

class B G protein-coupled receptor. The N-terminal 1 – 34 amino acids of PTH and PTHrP are sufficient for receptor activation. However, each region of PTHrP exhibits unique biological activities, likely acting through its own receptors. Although PTHrP circulates in some cancer patients and interacts with PTH/PTHrP receptors in bones and kidneys to cause hypercalcemia, the peptide does not normally circulate in an appreciable amount. Therefore, PTHrP is considered a local regulatory factor near its site of production rather than a classical circulating hormone. The peptide and its mRNA are ubiquitously expressed in many mature and developing tissues. The various isoforms have been shown to regulate many cellular functions such as myorelaxation, calcium transport, cell growth, and differentiation, indicating that PTHrP plays fundamental roles in the development and function of many tissues.

PTHrP gene expression is induced rapidly and can be altered by a number of factors such as TGF-β and serum. TGF-β stimulates the expression of PTHrP in hepatocytes and inhibits cell proliferation through a PTHrP-dependent mechanism. Overexpression of PTHrP enhances apoptosis in intestinal epithelial cells following serum depletion; mutation of the nuclear localization signal abolishes its ability to promote apoptosis by serum withdrawal. Functional linkage has been established between TGF-β and PTHrP during bone formation and bone metastasis by certain cancers. For example, TGF-β stimulates PTHrP expression in

bone culture and inhibits endochondral bone formation including condrocyte proliferation, hypertrophic differentiation, and matrix mineralization. The majority of breast cancers metastasizing to bone secrete PTHrP, which induces local osteolysis that leads to activation of bone matrix-derived TGF-β . In turn, TGF-β stimulates PTHrP expression, thereby accelerating bone destruction. These data suggest that PTHrP mediates certain biological effects of TGF-β .

b. The association of NF –KB with diabetic nephropathy

Diabetic nephropathy, one of the most serious microvascular complications of diabetes, is the leading cause of end-stage renal failure. The pathologic changes of diabetic nephropathy are characterized by early glomerular hypertrophy and later glomerulosclerosis and tubulointerstitial fibrosis, which caused by the accumulation of extracellular matrix (ECM) components in the glomerular mesangium and tubulointerstitium. Fibronectin, one of the important ingredients of ECM, is often used as an index to evaluate the extent of matrix accumulation. Therefore, inhibition of fibronectin production could be an effective strategy to delay glomerular sclerosis and prevent the progression of diabetic nephropathy.

The pathogenesis of diabetic nephropathy has not been fully elucidated. It is believed that diabetic nephropathy is a multifactorial process including dysregulated lipid and glucose homeostasis,

hemodynamic abnormalities, elevated oxidative stress, and hyperactive polyol and mitogen-activated protein kinase (MAPK) pathway activation. Growing evidences demonstrate that activation of nuclear factor-kappa B (NF-kB) and subsequently coordinated expression of gene products may play an important role in the pathogenesis of diabetic nephropathy. Nuclear factor-kB plays an important role in cell survival and its inhibition leads to apoptosis. Increased monocyte NF-kB activity seen in diabetics with nephropathy than diabetics without nephropathy. In vitro studies have demonstrated that high glucose, AGEs, AGN II, and stretch potently induce NF-kB activation mainly via formation of ROS and activation of PKC71 – 73 providing potential cellular mechanisms of NF-kB activation in the diabetic kidney. Recent studies have shown that NF-kB mediates both stretch and high glucose-induced monocyte hemoattractant protein (MCP) production in mesangial cell playing a role in glomerular epithelial cell apoptosis and modulates the TGF-β 1 intracellular signaling pathway. There is thus preliminary evidence for a role of NF kB in the pathogenesis of both glomerular and tubular damage in diabetes. Both ACE-inhibitor and statins are potent NF-kB inhibitors, and their renoprotective action, may be, at least in part, related to the suppression of NF-kB activity. It is also believed that diabetic nephropathy is one kind of chronic inflammation. In diabetes, the activated NF-kB translocates into the nucleus and triggers the expression

of its target genes including intercellular adhesion molecule-1 (ICAM-1) and transforming growth factor-beta 1 (TGF-β 1), which in turn induce persistent and enhanced inflammation, leading to excessive fibronectin production and ECM accumulation.

c. The association of MAPK, PI3K/Akt with diabetic nephropathy

Diabetic nephropathy, one of its microvascular complications, is also increasing markedly and has become a major cause of end stage renal disease. p38 mitogen-activated protein kinase (p38 MAPK) is a member of the mitogen-activated protein kinase family and is know a “stress-activated kinase” along with the c-Jun-NH2-terminal kinase. p38 MAPK is activated by varied environmental stressors, including osmolality changes oxidants, and proinflammatory cytokines, leading to cellular growth, differentiation, and apoptosis. In the situation of diabetes, nonenzymatic glycosylation of protein, polyalcohol pathway, diacylglycerol-protein kinase C pathway, and oxidative stress could activate p38 MAPK, resulting in the phosphorylation of transcriptional factor and alteration of expression of genes, which participated the development of diabetic nephropathy. The studies in vitro have shown that high level of glucose can activate p38 MAPK signaling pathway in renal cells and induce the phosphorylation of p38 MAPK in mesangial cells which promotes themesangial cells producing fibronectin. Therefore,

it is believed that p38 MAPK is a signaling transductor in the diabetic nephropathy and the agents that inhibit the activation of p38 MAPK signal pathway should reduce the formation of extracellular matrix in glomerular mesangium and block the thickening of glomerular basement membrane, preventing development of diabetic nephropathy.

An increased flux of glucose through the hexosamine pathway has also been linked to mechanisms of DN, particularly an increase in TGF-β . Fructose-6-phosphate from glycolysis is converted to glucosamine-6-phosphate in this pathway. Glycosylation of a transcription factor such as Sp1 by N-acetylglucosamine stimulates TGF-β transcription. In addition, an increase in flux through the hexosamine pathway up-regulates the expression of up-stream stimulatory factors(USFs) which transactivate the TGF-β 1 promoter. Intracellular accumulation of glucose also increases de novo formation of diacylglycerol (DAG) from glycolytic intermediates such as dihydroxyacetone phosphate. An increase in DAG activates several isoforms of PKC. Inhibition of PKC-β , the major isoform-induced in the kidney by hyperglycemia, ameliorates DN. Moreover, activation of PKC could, in turn, further stimulate MAPKs. Erk 1, 2 as well as p38 MAPK have been implicated as signaling intermediates in DN. MAPKs are additionally activated by ROS and there is likely cross-talk between the various pathways.

The serine/threonine kinase Akt regulates a number of cellular

functions, including glucose metabolism, glycogen synthesis, protein synthesis, cell proliferation, cell hypertrophy, and cell death, which is one of the downstream effectors of phosphoinositide 3-kinase (PI3K). It's demonstrated that Akt is an important mediator of mesangial cell proliferation and ECM protein accumulation. We also reported that Akt is activated in renal damage in streptozotocin-induced diabetic mice. The mitogen-activated kinases (MAPKs) are also unregulated in renal cells by hyperglycemia.

Studies have shown that the activation of p38 and extracellular signaling kinase (Erk) 1/2 are participating in renal tubular cells and mesangial cells proliferation in high glucoseSchizandrin (Sch), the major lignan isolated from the Schisandra chinensis, possesses many biological properties including hepatoprotective, anti-inflammatory, antitumor, and anti-asthmatic activities. In the kidney, Sch have shown the protective effect on gentamicin-induced nephrotoxicity against oxidative stress. It seems to exist on a mutual regulation between NADPH oxidase activation and PI3/Akt activation. Furthermore, the results indicate that NADPH oxidase and PI3K/Akt signaling activation is required for HG-induced Erk1/2 andP38 MAPK phosphorylation in mesangial cells. These data were correlated with inhibition effect on HG-mediated proliferation, protein synthesis, and ECM protein accumulation by Sch. The signaling molecules also are involved in HG-induced ECM protein overproduction.

Sch treatment prevents proliferation and protein synthesis as well as ECM protein accumulation by blocking NADPH oxidase activation mechanisms, which are ROS generation, Akt, and MAPK activation, under hyperglycemic condition. It does not exclude participation of other signaling pathways or complementary signaling within the presented diagram. Sch may become a valuable therapeutic drug candidate for treatment of diabetic nephropathy, particularly for targeting NADPH activation.

Vascular endothelial growth factor (VEGF) is one of the major factors promoting diabetic complications . VEGF is an endothelial mitogen and potent vasopermeability factor, the effects of which are mediated by endothelial cell-specific receptors . A recent study indicated that an antibody against VEGF treatment decreased hyperfiltration, albuminuria and glomerular hypertrophy in diabetic rats. These findings suggested that VEGF plays important roles in the pathogenesis of diabetic nephropathy and that VEGF may be a good therapeutic target molecule for diabetic nephropathy.

It is generally accepted that levels of VEGF and its receptor are increased in the kidneys of diabetic rats with glomerular lesions. Since podocytes are the major producers of VEGF within the glomeruli and podocyte injury underlies progressive glomerulosclerosis in diabetes both in humans and experimental models, regulation of VEGF expression in

the podocytes may provide novel insight into the pathogenesis of diabetic nephropathy. the present study investigated the effects of high glucose on VEGF production in podocytes. In addition, it’s reported here that high glucose induces VEGF expression through PKC and ERK pathways in podocytes.

d. The association of JAK/STAT with diabetic nephropathy

Recent studies suggest that Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling cascades may contribute to diabetic nephropathy. This pathway is mainly related to renal cell growth, production of the cytokine, TGF-b, as well as the ECM proteins collagen IV and fibronectin. Wang reported that the activation of JAK2 and STAT1 proteins was a requirement for the hyperglycemia-induced production of TGF-b and fibronectin in rat glomerular mesangial cells. JAK/STAT pathway is an essential intracellular mechanism of cytokine actions and constitutes a link between activation of cell surface receptors and nuclear transcriptional event. Control of the magnitude and duration of cytokine signaling is essential to prevent tissue damage. In this sense, recent studies have shown that JAK/STAT signaling can be regulated at many steps through different mechanisms. The suppressors of cytokine signaling (SOCS) proteins have defined an important additional mechanism for the negative regulation of the JAK/STAT pathway.

It’s demonstrated that overexpression of SOCS-1 in human

glomerular mesangial cells suppresses HG-induced JAK2/STAT activation and overproduction of TGF-b1 and fibronectin. The JAK/STAT pathway is an important link between cell surface receptors and nuclear transcriptional events leading to cell growth. The JAK/STAT pathway, especially the JAK2 – STAT1-dependent pathway, contributes to HG stimulating TGF-b and fibronectin production in cultured rat kidney glomerular mesangial cells. Therefore, it appears that the activation of JAK2 and STAT proteins by hyperglycemia might play an important role in both promoting cell proliferation and the synthesis of ECM molecules. Thus the activation of JAK/STAT may be one of the major mechanisms involved in high glucose-induced glomerular injury. The blockade of JAK/STAT may be an effective method to therapy diabetic nephropathy.

2. Oxidative stress

Increase in oxidative stress and the overproduction of reactive oxygen species (ROS) in diabetes is occurring due to hyperglycemia. This ROS induces peroxidation of cell membrane lipids, oxidation of proteins, renal vasoconstriction and deoxyribonucleic acid (DNA) damage. Various biochemical pathways are also stimulated through the increased generation of ROS mainly PKC pathways, AGE formation, TGF-β , and ANG-II.

Hyperglycemia results in an increase in mitochondrial ROS

formation. An increase in glucose uptake leads to overproduction of electron donors (NADH and FADH 2) from stimulated glycolysis and the tricarboxylic acid cycle. At the mitochondrial inner membrane, where the electron transport chain is localized, the increase in electron donors (NADH, FADH 2) generates a high membrane potential by pumping protons across the inner membrane. As a consequence, electron transport is inhibited at complex III increasing the half-life of free-radical intermediates of coenzyme Q, which finally reduces O2 to superoxide.

3. Advanced glycation end products

In longstanding hyperglycemia, the excess glucose combines with free aminoacids or tissue proteins. This glycosylation leads to the development of DN. This process initially forms reversible early glycosylation products and later irreversible AGE. The matrix proteins in the glomerular epithelial cells get accumulated along with decrease in collagenase activity and defect in the glomerular epithelial cell tight junction, because of the increase in AGEs.

Conclusion

Diabetic nephropathy develops due to the combined action of both hemodynamic and signal pathways. Signal pathways are also activated within the diabetic kidney and result in accumulation of AGEs, activation of PKC, renal polyol formation and enhanced oxidative stress. These derangements activate various cytokines and growth factors. To improve

the outcome of DN, more scientific knowledge is needed.

References

1. Guoguo Shang, Xinjun Tang, Pan Gao, Fanli Guo, Hongpeng Liu, Zhonghua Zhao, Qi Chen, Tao Jiang. Sulforaphane attenuation of experimental diabetic nephropathy involves GSK-3 beta/Fyn/Nrf2 signaling pathway. Journal of Nutritional Biochemistry 26 (2015) 596 – 606.
2. Eunsoo Jung, JunghyunKim, SungHoKim, SanghwaKim, Myung-Haing Cho. Gemigliptin improves renal function and attenuates podocyte injury in mice with diabetic nephropathy. European Journal of Pharmacology 761 (2015) 116–124.
3. Wei-Jian Ni, Hai-Hua Ding, Li-Qin Tang. Berberine as a promising anti-diabetic nephropathy drug: An analysis of its effects and mechanisms. European Journal of Pharmacology 760 (2015)103–112.
4. Fang Yao, Zhen Li, Takashi Ehara, Lin Yang, DandanWang, Lulu Feng, Yiming Zhang, KunWang, Yonghong Shi, Huijun Duan, Lianshan Zhang. Fatty Acid-Binding Protein 4 mediates apoptosis via endoplasmic reticulum stress in mesangial cells of diabetic nephropathy. Molecular and Cellular Endocrinology 411 (2015) 232 – 242.
5. Chia-Wen Kuo, Chih-Jie Shen, Yu-Tang Tung, Hsiao-Ling Chen, Yu-Hsuan Chen, Wen-Hui Chang, Kai-Chung Cheng, Shang-Hsun

Yang, Chuan-Mu Chen. Extracellular superoxide dismutase ameliorates streptozotocin-induced rat diabetic nephropathy via inhibiting the ROS/ERK1/2 signaling. Life Sciences xxx (2015) xxx–xxx.

6. Yanna Cao, Weili Zhang, Xuxia Gao, Guohua Zhang, Miriam Falzon, Courtney M. Townsend Jr., Mark R. Hellmich, Tien C. Ko. PTHrP is a novel mediator for TGF-β -induced apoptosis. Regulatory Peptides 184 (2013) 40–46.
7. Yue Zhang, Hongbo Li, Jun Hao, Yi Zhou, Wei Liu. High glucose increases Cdk5 activity in podocytes via transforming growthfactor-β 1 signaling pathway. EXPERIMENTAL CELL RESEARCH 326 (2014) 219 – 229.
8 Weihua Liu , Xiaoyan Zhang, Peiqing Liu, Xiaoyan Shen, Tian Lan, Wenyuan Li, Qin Jiang, Xi Xie, Heqing Huang. Effects of berberine on matrix accumulation and NF-kappa B signal pathway in alloxan-induced diabetic mice with renal injury. European Journal of Pharmacology 638 (2010) 150–155.
9. Jinping Wang, Heqing Huang, Peiqing Liu, Futian Tang, Jia Qin, Weng Huang, Fengying Chen, Fenfen Guo, Weihua Liu, Bing Yang. Inhibition of phosphorylation of p38 MAPK involved in the protection of nephropathy by emodin in diabetic rats. European Journal of Pharmacology 553 (2006) 297–303.

10. Dan Zhang, ShiyingShao, HongxiaShuai, YunchuanDing, WeiShi, DaowenWang, XuefengYu, SDF-1α reduces fibronectin expression in rat mesangial cells induced by TGF-β 1 and high glucose through PI3K/Akt pathway. EXPERIMENTAL CELL RESEARCH 319 (2013) 1796 – 1803.
11. Sachi Hoshi, Ken-ichi Nomoto, Junro Kuromitsu, Shinsuke Tomari, and Michio Nagata. High Glucose Induced VEGF Expression via PKC and ERK in Glomerular Podocytes. Biochemical and Biophysical Research Communications 290 (2002) 177–184.
12. Seung-I Jeong, Sang-Jun Kim, Tae-Ho Kwon, Kang-Yeol Yu, Seon-Young Kim. Schizandrin prevents damage of murine mesangial cells via blocking NADPH oxidase-induced ROS signaling in high glucose. Food and Chemical Toxicology 50 (2012) 1045 – 1053.
13. Ting He, Xu Guan, SongWang, Tangli Xiao, Ke Yang, Xinli Xu, JunpingWang, Jinghong Zhao. Resveratrol prevents high glucose-induced epithelial – mesenchymal transition in renal tubular epithelial cells by inhibiting NADPH oxidase/ROS/ERK pathway. Molecular and Cellular Endocrinology 402 (2015) 13 – 20.
14. Jau-Shyang Huang, Chao-Tang Chuang, Mei-Hsin Liu, Sheng-Hsuan Lin, Jinn-Yuh Guh, Lea-Yea Chuang. Klotho attenuates high glucose-induced fibronectin and cell hypertrophy via the ERK1/2-p38 kinase signaling pathway in renal interstitial fibroblasts. Molecular

and Cellular Endocrinology 390 (2014) 45 – 53.

15. Yonghong Shia, Yanling Zhang, Chen Wang, Chunyang Du, Song Zhao, Zhao Qi, Qingxian Zhang, Huijun Duan. Suppressor of cytokine signaling-1 reduces high glucose-induced TGF-b1 and fibronectin synthesis in human mesangial cells. FEBS Letters 582 (2008) 3484 – 3488.
16. Motohide Isono, Sheldon Chen, Soon Won Hong, M. Carmen Iglesias-de la Cruz, and Fuad N. Ziyadeh. Smad pathway is activated in the diabetic mouse kidney and Smad3 mediates TGF-b-induced fibronectin in mesangial cells. Biochemical and Biophysical Research Communications 296 (2002) 1356 – 1365.
17. Yasushi Hirasawa, Takayuki Sakai, Masanori Ito, Hiromitsu Yoshimura, Yibin Feng, Tadashi Nagamatsu. Advanced-glycation-end-product-cholesterol-aggregated-protein accelerates the proliferation of mesangial cells mediated by transforming-growth-factor-beta 1 receptors and the ERK-MAPK pathway. European Journal of Pharmacology 672 (2011) 159 – 168.
18. Wei Zhang, Shangxin Song, Fei Liu, Yi Liu, Yuanshu Zhanga. Beta-casomorphin-7 prevents epithelial-mesenchymal transdifferentiation of NRK-52E cells at high glucose level: Involvement of AngII-TGF-β 1 pathway. Peptides 70 (2015) 37–44.
19. Chi-Hsien Chou, Lea-Yea Chuang, Chi-Yu Lu, Jinn-Yuh Guh.

Vitamin D-binding protein is required for the protective effects of vitamin D in renal fibroblasts and is phosphorylated in diabetic rats. Molecular and Cellular Endocrinology 411 (2015) 67 – 74.

20. Anaıs Mozar, Nagesha Guthalu Kondegowda, Ilana Pollack, Rafael Fenutria, Rupangi C. Vasavada. The Role of PTHrP in Pancreatic Beta-Cells and Implications for Diabetes Pathophysiology and Treatment. Clinic Rev Bone Miner Metab 12 (2014) 165–177.

对氧磷酯酶 1（PON1）与动脉粥样硬化

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摘要：动脉粥样硬化是一种累及大中动脉的慢性性进行性疾病，已严重危害人类的健康，其发病机制较复杂，血脂代谢紊乱和氧化应激是动脉粥样硬化发生和发展的两个重要危险因素。高密度脂蛋白是公认的抗动脉粥样硬化因素，对氧磷酶 1（PON1）是高密度脂蛋白重要组成成分之一，在高密度脂蛋白抗炎、抗氧化、介导的胆固醇外流过程中发挥了重要作用。众多临床性和实验性数据表明，PON1 通过不同机制对动脉粥样硬化起到了一定抑制作用。本文对对氧磷酶 1 及动脉粥样硬化的关系和作用作一综述。

前言：动脉粥样硬化（atherosclerosis,AS）是以血管壁脂质及炎性介质聚集为主要病理特征的慢性进行性疾病，是全球导致死亡的头号杀手。其原因可能是巨噬细胞、平滑肌细胞或内皮细胞在氧化应激状态下产生大量的活性氧化脂质，进一步损伤血管内皮细胞，促进血管平滑肌细胞的增殖，刺激巨噬细胞的泡沫化，从而导致动脉粥样硬化斑块的形成。[1]目前，动脉粥样硬化导致的心脑血管疾病已严重危害到人类健康，是全球导致死亡的头号杀手。因此，探讨与动脉粥样硬化的发生发展及防治相关的各种因素有重要意义。

对氧磷酶 1（paraoxonase1，PON1）PON1 是一类钙依赖性与高密度脂蛋白（high density lipoprotein ，HDL）相关的内酯酶，能水解多种底物，如有机磷、内酯、芳香酯和氧化磷脂等。由肝脏合成和分泌，并与 HDL 紧密结合，分布于血液和肝脏。近年来，有大量研究表明，PON1 的含量与活性与多种疾病有关，如心脑血管疾病、有机磷中毒糖尿病和相关的肾脏疾病、眼部疾病以及帕金森病等。PON1 作为高密度脂蛋白的重要组分，在康动脉粥样硬化过程中起着非常重要作用。本文结合部分文献，对 PON1 的来源和结构、PON1 抗动脉粥样硬化的机制、PON1 的多态性与动脉粥样硬化的关系做一综述。

有研究表明，PON1 抗动脉粥样硬化的特性是与其能够保护 LDL、HDL 不受氧化，减少巨噬细胞的氧化应激状态[2]，刺激胆固醇从巨噬细胞外流[3]，减少动脉粥样样硬化损伤的氧化状态[4]有关。其次，PON1 被认为有抗炎作用，通过生物灭火 ox-LDL 中毒性脂质来减少血管壁细胞的氧化应激应答。进一步有研究认为，PON1 减少单核细胞向内皮细胞的趋化和粘附，抑制了单核细胞向巨噬细胞的分化。PON1 有多种基因多态性位点，这些基因多态性引起基因启动子活性的差异，导致基因表达水平和 PON1 活性的差异，从而影响动脉粥样硬化的形成。

关键词：对氧磷酶 1，动脉粥样硬化，脂蛋白代谢，氧化应激

1 PON1 的来源

众多研究证实，高同型半胱氨酸（Hcy）血症是动脉粥样硬化的独立危险因素，但 Hcy 并不直接导致动脉粥样硬化的形成，但是其活性很高的代谢产物同型半胱氨酸硫内酯（HTL）可以导致动脉粥样硬化。Hcy 在蛋氨酸-tRNA 合成酶催化下形成同型半胱氨酸硫内酯。HTL 与蛋白中的赖氨酸结合，导致蛋白质被同型半胱氨酸化，从而损伤蛋白的正常生理功能，导致细胞死亡、自身免疫反应、炎症反应，导致动脉粥样硬化。HTL 在同型半胱氨酸硫内酯酶（HTase）作用下又可以水解为 Hcy，而 HTase 就是所谓的 PON1。PON1 基因位于 7q21.3-7q22.1，含有九个外显子及八个内含子。PON1 是一个含 355 氨基酸残基的糖蛋白，分子量在 43kDa-45kDa 之间，由 6 个圆柱状的β 螺旋结构组成，其中 4 个β 链连接的二硫桥，稳定了酶的结构。PON1 在 42、284、353 包含三个半胱氨酸残基，其中 284 位点的残基有一个游离的巯基，这与酶的保护作用有关。载脂蛋白 apoA-I 使 HDL 中 PON1 更加稳定并且选择性提高了 PON1 的内酯酶活性。[5]

PON 基因家族有三个成员 PON1、PON2、PON3，这三个基因彼此相连。PON1 和 PON3

主要存在血浆中，与 HDL 相连。PON2 主要分布在细胞内。在动脉粥样硬化进程中，PON1、PON2、PON3 都可以延缓 LDL 氧化。

2 PON1 抗动脉粥样硬化的机制

有研究[6]认为，PON1 是通过两条途径共同来延缓动脉粥样硬化进程。一是作为 HTase 可以保护蛋白质免受同型半胱氨酸化。二是作为抗氧化剂保护 LDL、HDL 等脂蛋白免受氧化修饰。目前 PON1 发挥动脉粥样硬化保护作用的机制主要包括减少巨噬细胞自身氧化及其氧化 LDL 的能力、抑制胆固醇合成、促进胆固醇外流。具体机制如下：

2.1 PON1 保护 HDL 和 LDL 免被氧化

PON1 是 HDL 的重要组分之一，有研究表明，增加 HDL 中的 PON1 的含量并不能改变 HDL 的成分或性质，但是可以增强其抗脂质氧化的能力。有报道称，PON1 并不是 HDL 中的固定组分，因为 PON1 能以具有酶催化活性的形式，从 HDL 转移到细胞质膜的外表面从而发挥其功能。[7]因此，PON1 对维持 HDL 的完整性及功能有重要意义。氧化的 LDL 对于血管内皮细胞、血管平滑肌细胞、巨噬细胞均有毒性作用，而正常功能的 HDL 可以将这些毒性作用清除。目前已经证实有四种参与了 HDL 的抗氧化机制，即胆固醇酰基转移酶（LCAT）、胆固醇酯转换蛋白（CETP）、对氧磷酶 1（PON1）、脂蛋白相关磷脂酶 A（Lp-PLA2）。这四种酶协同 HDL 其他相关蛋白共同阻止或延缓 LDL 氧化。

2.2 PON1 减少巨噬细胞的炎症应答。

Saar Aharoni 等人[8]以 C57BL/6 和 human-PON1 transgennic 小鼠的骨髓巨噬细胞为研究对象，PON1 处理后测炎症的表达水平。结果表明，PON1 减弱了 M1 型诱导的 ROS 的产生，吞噬作用及巨噬细胞的坏死。并且 PON1 的抗炎特性是由 SR-BI 而非 ABCA1 介导的。Bianca Fuhrman 等人[9]以 PON1 敲除小鼠的腹腔巨噬细胞为研究对象，结果表明小鼠体内 PON1 的缺乏是和巨噬细胞减少的 SR-BI 表达、减少的细胞内与 HDL 的结合和 HDL 介导的细胞保护作用的丧失有关。

2.3 PON1 可以抑制胆固醇的生物合成

胆固醇的积累和泡沫细胞的形成是动脉粥样硬化早期标志之一。有研究表明，PON1 能有效抑制巨噬细胞内胆固醇的合成，PON1 基因敲除的小鼠细胞胆固醇含量明显增加。[10]PON1 能抑制胆固醇的生物合成，一方面是因为 PON1 可以通过功能区下游的甲羟戊酸直接抑制胆固醇的合成，另一方面可能是由于 PON1 磷脂酶 A2 的活性，磷脂酶 A2 能促进融血磷脂胆碱（LPC）的形成，而 LPC 能激活一些信号转导途径抑制胆固醇的生物合成[11]。PON1 不仅对各种类型的巨噬细胞的胆固醇合成有抑制作用，对血管内皮细胞和肝脏细胞也有这种作用。

2.4PON1 具有同型半胱氨酸内酯酶活性，能够降低 HTL 水平。

同型半胱氨酸浓度升高是动脉粥样硬化性血管疾病风险增加的危险独立因素。而 HTL 是真正导致动脉粥样硬化的毒性代谢产物。

3 PON1 基因多态性

目前通过基因测序发现 PON1 基因多态性位点大约有 200 种，其中位于 5’端非翻译区的有 7 个，位于内含子区域的 171 个，位于 3’端非翻译区的有 15 个。编码区常见的 2 种基因多态性 192Q/M，55L/M，即 192 位点谷氨酸（Glu，Q）与精氨酸（Arg，M）置换，55 位点亮氨酸(Leu,L)与蛋氨酸(Met,M)置换。启动子区域确定了 5 种基因多态性：-108C/T，-126G/C，-162G/A，-831G/A，-909G/C。这些多态性引起基因启动子活性的差异，引起 PON1 基因表达水平的差异血浆 PON1 活性的差异。目前研究认为 192Q/R,55L/M,-162G/A,-909G/C,-107C/T 等与心血管等动脉粥样硬化疾病关系密切，其他基因多态性尚未证实有疾病相关性。

4 PON1 基因多肽性及与动脉粥样硬化

大量研究发现，在高胆固醇血症、糖尿病、冠心病等动脉粥样硬化疾病患者中 PON1 的

活性是较健康人低的。故PON1的活性与动脉粥样硬化呈负相关。用免疫法标记PON1后发现，在大动脉中PON1的浓度随着动脉粥样硬化的进展而逐渐增加。并且在动脉巨噬细胞中PON1和PON3的出现都提示了细胞保护作用[12]。最近一项有关PON1活性和冠心病敏感性关系的研究，对9854例冠心病患者和11408例健康对照者进行比较分析，发现冠心病患者组比健康对照组PON1的活性降低了19%（p<10-5）;同样在冠脉狭窄、心肌梗死的病例的分析中也得出了同样的结果[13]。低PON1的浓度还预示透析患者中心血管的死亡率[14]。

目前很多研究已证实，PON1基因多态性与心血管疾病、动脉粥样硬化、冠心病及2型糖尿病密切相关。Meta分析，PON1-192Q/R多态性是CHD的危险因素。国内有研究[15, 16]证实，PON1第55位基因位点与脑梗塞合并高血压有关，M等位基因是高血压并发动脉粥样硬化脑梗塞的危险因素之一。262位T等位基因携带者易患大动脉粥样样硬化脑梗塞，标志着T等位基因可能是其的一个遗传标志。有研究[17]通过对脑梗患者PON1基因启动子区-909G/C基因多态性分布频率进行分析，结果显示基因型为GG、CC、GC、G等位基因。病例组基因型GG、G频率均显著高于对照组。同时多基因Logistics分析，G等位基因是脑梗塞的易感因素，基因型GG可能是其危险因素。目前共识的是，-107C/T多态性与PON1的表达密切相关。然而，在PON1活性相关的单核苷酸基因多态性全基因研究中发现，尽管基因多态性与血清PON1活性相关,但基因型却与MEAE事件的发生无明显的相关性[18](P>0.05)。目前这种差别的原因尚不明确，但是反映出这样一个结论，PON1在血清中和动脉壁中的活性是不同的。PON1的基因型影响的是血清中PON1的活性，而真正发挥抗动脉粥样硬化作用的是动脉壁中PON1的水平[19]。

综上所诉，PON1可以通过抑制脂蛋白LDL、HDL的氧化，抑制巨噬细胞像泡沫细胞的分化，减少巨噬细胞的炎症应答及凋亡坏死来延缓或抑制动脉粥样硬化进程。PON1的基因多态性影响了PON1基因启动子的活性，导致PON1的表达和活性差异，与脑卒中、动脉粥样硬化、冠心病、2型糖尿病密切相关。虽然有大量实验数据证明PON1有抗动脉粥样硬化的作用，但仍需要大量的基础及临床研究来证实PON1与动脉粥样硬化性疾病的相关性，从而为预防、诊断、临床提供重要的意义。

1. Brinkley TE, Nicklas BJ, Kanaya AM, Satterfield S, Lakatta EG, Simonsick EM, Sutton-Tyrrell K, Kritchevsky SB: **Plasma Oxidized Low-Density Lipoprotein Levels and Arterial Stiffness in Older Adults: The Health, Aging, and Body Composition Study**. *Hypertension* 2009, **53**(5):846-852.
2. Rozenberg O, Shih DM, Aviram M: **Paraoxonase 1 (PON1) attenuates macrophage oxidative status: studies in PON1 transfected cells and in PON1 transgenic mice**. *Atherosclerosis* 2005, **181**(1):9-18.
3. Berrougui H, Loued S, Khalil A: **Purified human paraoxonase-1 interacts with plasma membrane lipid rafts and mediates cholesterol efflux from macrophages**. *Free radical biology & medicine* 2012, **52**(8):1372-1381.
4. Tavori H, Vaya J, Aviram M: **Paraoxonase 1 attenuates human plaque atherogenicity: relevance to the enzyme lactonase activity**. *Advances in experimental medicine and biology* 2010, **660**:99-111.
5. Gaidukov L, Tawfik DS: **High affinity, stability, and lactonase activity of serum paraoxonase PON1 anchored on HDL with ApoA-I**. *Biochemistry* 2005, **44**(35):11843-11854.
6. Rodriguez-Sanabria F, Rull A, Beltran-Debon R, Aragones G, Camps J, Mackness B, Mackness M, Joven J: **Tissue distribution and expression of paraoxonases and**

chemokines in mouse: the ubiquitous and joint localisation suggest a systemic and coordinated role. *Journal of molecular histology* 2010, **41**(6):379-386.

7. Deakin SP, Bioletto S, Bochaton-Piallat ML, James RW: **HDL-associated paraoxonase-1 can redistribute to cell membranes and influence sensitivity to oxidative stress**. *Free radical biology & medicine* 2011, **50**(1):102-109.
8. Aharoni S, Aviram M, Fuhrman B: **Paraoxonase 1 (PON1) reduces macrophage inflammatory responses**. *Atherosclerosis* 2013, **228**(2):353-361.
9. Fuhrman B, Gantman A, Aviram M: **Paraoxonase 1 (PON1) deficiency in mice is associated with reduced expression of macrophage SR-BI and consequently the loss of HDL cytoprotection against apoptosis**. *Atherosclerosis* 2010, **211**(1):61-68.
10. Rozenberg O, Shih DM, Aviram M: **Human serum paraoxonase 1 decreases macrophage cholesterol biosynthesis: possible role for its phospholipase-A2-like activity and lysophosphatidylcholine formation**. *Arteriosclerosis, thrombosis, and vascular biology* 2003, **23**(3):461-467.
11. Rosenblat M, Hayek T, Hussein K, Aviram M: **Decreased macrophage paraoxonase 2 expression in patients with hypercholesterolemia is the result of their increased cellular cholesterol content: effect of atorvastatin therapy**. *Arteriosclerosis, thrombosis, and vascular biology* 2004, **24**(1):175-180.
12. Marsillach J, Camps J, Beltran-Debon R, Rull A, Aragones G, Maestre-Martinez C, Sabench F, Hernandez M, Castillo DD, Joven J *et al*: **Immunohistochemical analysis of paraoxonases-1 and 3 in human atheromatous plaques**. *European journal of clinical investigation* 2011, **41**(3):308-314.
13. Wang M, Lang X, Cui S, Zou L, Cao J, Wang S, Wu X: **Quantitative assessment of the influence of paraoxonase 1 activity and coronary heart disease risk**. *DNA and cell biology* 2012, **31**(6):975-982.
14. Ikeda Y, Suehiro T, Itahara T, Inui Y, Chikazawa H, Inoue M, Arii K, Hashimoto K: **Human serum paraoxonase concentration predicts cardiovascular mortality in hemodialysis patients**. *Clinical nephrology* 2007, **67**(6):358-365.
15. **<对氧磷酶基因多态性与动脉粥样硬化性脑梗死的关联_王燕飞.pdf>**.
16. **<对氧磷酶 1 基因 262 位点 C_T 多态性与脑梗死的关联性分析_何娜.pdf>**.
17. Younis NN, Soran H, Charlton-Menys V, Sharma R, Hama S, Pemberton P, Elseweidy MM, Durrington PN: **High-density lipoprotein impedes glycation of low-density lipoprotein**. *Diabetes & vascular disease research* 2013, **10**(2):152-160.
18. Koren-Gluzer M, Aviram M, Meilin E, Hayek T: **The antioxidant HDL-associated paraoxonase-1 (PON1) attenuates diabetes development and stimulates beta-cell insulin release**. *Atherosclerosis* 2011, **219**(2):510-518.
19. Mackness M, Mackness B: **Targeting paraoxonase-1 in atherosclerosis**. *Expert opinion on therapeutic targets* 2013, **17**(7):829-837.

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清道夫受体 B 类Ⅰ型与动脉粥样硬化

摘要：清道夫受体 B 类Ⅰ型是高密度脂蛋白受体，主要分布于肝脏和生成类固醇激素的组织。它与高密度脂蛋白以高亲和性结合，还可与天然的低密度脂蛋白、乙酰化低密度脂蛋白、氧化低密度脂蛋白、磷脂酰丝氨酸、阴离子磷脂等结合，对胆固醇和胆固醇酯的代谢起着重要的作用，可影响胞质膜胆固醇的分布，改变膜结构，从而影响多种生理和病理生理功能。

关键词：清道夫受体 B 类Ⅰ型；高密度脂蛋白；胆固醇；动脉粥样硬化

动脉粥样硬化(atherosclerosis， As)是一种常见病、多发病，可引起多种严重的心脑血管疾病，其特点是动脉发生了非炎症性、 退行性及增生性改变， 导致管壁增厚变硬，弹性消失、管腔缩小，主要与脂质代谢异常有关.AS 的特征之一是粥样斑块中存在含脂类的泡沫细胞，而巨噬细胞源性泡沫细胞又是斑块中泡沫细胞的主要成分。动脉粥样硬化清道夫受体 B 类Ⅰ型(scavenger receptorclass B typeⅠ , SR -BⅠ)对高密度脂蛋白(high density lipopro -tein,HDL) 介导的胆固醇和胆固醇酯的摄取起着重要的调节作用，它可促进“ 胆固醇的逆向转运”，清除血及组织中过多的胆固醇，保护血管，防止动脉粥样硬化的形成或改善已形成的动脉粥样硬化[1] 。SR -BⅠ的表达可改变胞质膜上胆固醇的分布，导致新的膜结构的形成，这提示 SR -BⅠ 在细胞内有多种功能 [2, 3] 。

1 清道夫受体概况

SRB 包括 CD 36 和 SRB1 两种亚型。SR -BⅠ 在小鼠和大鼠的肝脏及生成类固醇激素的组织中高度表达，如肾上腺、睾丸、 卵巢。 在巨噬细胞、脑内的星形细胞、血管平滑肌细胞上都有表达。人类 SR -BⅠ 的表达分布与大鼠相似, SR -B Ⅰ 的组织分布适合于 HDL 受体。 它在肝脏和生成类固醇组织高水平表达，这些主要是高水平吸收 HDL 胆固醇酯的组织 [4] 。SRB1 另外也可与修饰的脂蛋白、 氧化的和乙酰化的 LDL、 磷脂酰丝氨酸、 阴离子磷脂、 凋亡细胞、 非修饰的 LDL 和 VLDL 等结合。

2 结构及可能的功能

SR -BⅠ 是位于细胞表面的一种糖蛋白，含 509 个氨基酸残基。 SR -BⅠ定位于人类第 12 号染色体上, 12q24. 2 -qter 位,基因长约 75kb，包括 13 个外显子和 12 个内含子。 成熟的 SR -BⅠ的相对分子质量约为 82×10 3 , 含 509 个氨基酸残基。SR -BⅠ 的拓扑结构呈马蹄形, N 末端和 C 末端位于胞质内，分别含 8 个和 45 个氨基酸残基，称为胞质域；紧接着 N 末端和 C 末端的分别是 28 个和 25 个氨基酸残基构成的跨膜域；细胞膜外，和两个跨膜域相连的是一个大的胞外域，由 403 个氨基酸残基构成[5] 。

SR -BⅠ 胞质域羧基端最后 15 个氨基酸与 PDZK1 作用。在中国仓鼠卵细胞上共表达 PDZK1 和 SR -BⅠ 可增加选择性吸收，并使 SR -B Ⅰ 蛋白水平也成比例地增加，但是 SR -BⅠmRNA 水平没有增加，这可能与 PDZK1 抑制 SR -BⅠ分解有关 [6] 。 SR -BⅠ 羧基端最后 3 个氨基酸为精氨酸-赖氨酸-亮氨酸，在人、小鼠、大鼠、牛、仓鼠中高度保守，去除最后一个氨基酸也不能使 SR -BⅠ 与 PDZK1 作用，但在选择性吸收中显示与野生型 SR -BⅠ 有相同的功能。去除羧基端最后一个氨基酸的 SR -BⅠ 转基因小鼠(SR -BⅠ del509)，其 mRNA 表达约是非转基因组的 60 倍，蛋白表达却只是非转基因组的 6 倍。 SR -BⅠ del509 小鼠血浆三酰甘油水平与非转基因小鼠的无差别。所以 , SR -BⅠ 的与 PDZK1 作用域是肝细胞表达 SR -BⅠ mRNA 所必需的，也是其发挥功能所必需的 [2] 。

研究表明, SR -BⅠ 胞外域紧靠 C 末端跨膜域的部分对 SR -BⅠ 调节自由胆固醇外流到磷脂颗粒和增加对胆固醇氧化酶敏感的膜自由胆固醇池是必需的。 此部位抗原抗体作用后, SR -BⅠ 不表达。R -BⅠ 胞外域 192、388 位点周围改变, 可使其表达较野生型降低 50%, 且不具有或者仅有很低的 HDL 结合能力。胞外域另有一些突变可使其表达有所降低, 与 HDL 的结合有所降低, 并使其失去对 HDL 胆固醇酯的选择性吸收 [7] 。

SR -BⅠ 定位于胆固醇丰富区域的功能意义是什么? 一种可能性是 SR -BⅠ 能形成其他蛋白发挥功能所必需的胞核部分。Uittenbogaard 等 [8] 表明, CD 36 调节的小凹陷处的胆固醇外移到氧化低密度脂蛋白, 导致内皮型一氧化氮合酶在细胞内膜上重新分布, 减少了乙酰胆碱诱导的内皮型一氧化氮合酶的活性, SR -BⅠ 与 CD 36 共同位于一个区域上, 可恢复内皮型一氧化氮合酶的分布, 恢复通过 HDL 胆固醇摄取流入小管的活性。所以, 暂且可这样推测, SR -BⅠ 可影响来自于胆固醇区域的信号转导途径的活性, SR -BⅠ 对内皮型一氧化氮合酶及同-raft 有关受体的生理意义可通过 SR -BⅠ 缺陷小鼠得到更好的理解。

3 SR -BⅠ的表达和调节

SRB1 的表达受促激素、 胆固醇、 修饰的 LDL、 缺氧等因素的调控。促激素通过 cAMP/蛋白激酶 A 信号转导途径对 SRB1 的表达进行调控;维生素 E 对 SRB1 表达则由蛋白激酶 C 所介导 [9- 10] 。胆固醇可以通过固醇调节元件结合蛋白 1a 发挥调控作用， 在载脂蛋白 A Ⅰ基因剔除鼠和卵磷脂胆固醇脂酰基转移酶基因剔除小鼠中， SRB1 在肾上腺的表达上调，说明 SRB1 表达受胆固醇代谢的影响。有研究发现， 胰岛素样生长因子 1 通过磷脂酰激醇 3- 激酶信号转导途径抑制 SRB1 启动子的活性进而下调 SRB1 的表达 [11] 。Svensson 等 [12] 的研究表明， 缺氧状态下， SRB1 在巨噬细胞的表达显著减少;暴露于修饰的 LDL 时 SRB1 的表达增加(即使是轻微程度的修饰)。此外， SRB1 亦受 PPARα 的调节。PPARγ 激活物作用于人巨噬细胞可诱导 SRB1 的表达。在载脂蛋白 E 缺乏小鼠的大动脉给予 PPAR 激活物可诱导 SRB1 表达。

在整体和离体,生成类固醇激素组织内 SR -BⅠ 的表达受营养激素的调节, 其信号转导途径为环磷腺苷蛋白激酶 A 途径, 涉及 CCAAT 增强子结合蛋白、类固醇激素生成因子。SR -BⅠ转录的调节与 SR -BⅠ增强子结合位点同一系列转录因子结合有关, 这些转录因子包括类固醇激素生成因子和固醇调节因子结合蛋白等。另外, Ikemoto 等 [5] 确定 PDZ 胞质域与 SR -BⅠ的 C 末端相互作用, 可影响其活性。

4 SR -B1 的生理功能与动脉粥样硬化(AS) 的关系

Acton 等认为 HDL 与 SR -BⅠ 的结合是由 HDL 中的主要载脂蛋白 apoAⅠ 、apoAⅡ和 apoA Ⅲ所介导 [13] 。 但是之后, Strangl 在实验中又发现, SR -BⅠ 所识别的并不是 HDL 中的蛋白, 而是脂类成分 [14] 。HDL 在外周组织与胆固醇结合, 并将其酯化为胆固醇酯, 以 HDL 胆固醇酯的形式运至肝脏、肾上腺、卵巢等组织。 大约 20 年前,Glass 等在血浆 HDL 的脂质和蛋白质组分研究中发现了胆固醇的选择性摄取。 当来自外周组织携带有胆固醇酯的 HDL 与肝脏以及类固醇生成组织细胞表面的 SR -BⅠ特异结合后,HDL 中的胆固醇酯从其疏水中心(而非表面的载脂蛋白) 被吸入细胞内.

SR -BⅠ表达的调节与胆固醇的代谢有关, HDL 具有抗 As 的作用， 其主要通过逆行转运胆固醇、 抗氧化、 抗炎症以及抗凝起作用。SR -BⅠ通过 “选择性摄取途径” 将 HDL 胆固醇酯传递给肝脏和类固醇激素生成组织， 即 SRB1 与 HDL 结合后， HDL 疏水核心的胆固醇酯交给浆膜而不伴有 HDL 完整颗粒的摄取和降解。HDL 中的 apoA2Ⅰ需要占据合适的位置以形成 “生产型复合物” ， 即一种介于 HDL 与 SRB1 之间的， 能导致有效的脂质摄取过程发生的复合物 [15] 。 SR -BⅠ与 HDL 一起对 As 性心血管疾病的发生起保护作用。已有研究证实， 减少小鼠 SRB1 的表达可使血浆总胆固醇的水平提高， SRB1 在肝内和肝外组织都能发挥抗 As 的作用 [16-17] 。

HDL 胆固醇, 作为胆汁胆固醇的 主要来源, 可能通过 SR -BⅠ 被肝脏摄取, 然后分泌入胆汁或转化为胆酸。在肝脏,SR -BⅠ 是怎样调节 HDL 来源的胆固醇酯和自由胆固醇的选择性吸收的机制还不清楚。SR -BⅠ 能够改变细胞质膜胆固醇的分布, 调节其他膜蛋白的活性, 提示 SR -BⅠ 在膜脂质的组成上可能起作用,

对其他过程可能有深远的影响.胆固醇酯通过 HDL 颗粒的核心直接进入细胞质膜，这是中性脂质离开 HDL 核心的机制。 在模型膜和脂蛋白上人们发现，胆固醇酯与胆固醇不一样，它在磷脂表面的溶解度很低，大部分进入颗粒的核心。 所以，人们推断整个 HDL 颗粒与 SR -BⅠ 结合，经 SR -BⅠ 介导进入到早期内涵体系统。 在内涵体，胆固醇酯分子经这一部位的中性胆固醇酯酶被水化，胆固醇进入内涵体膜。 HDL 颗粒分解出胆固醇酯和胆固醇，然后回到细胞质膜，分泌出细胞，胆固醇分泌入胆汁。 胆固醇酯的水化不是摄取所必需的，因为非水化胆固醇酯类似物和胆固醇酯可被选择性吸收，但是，与来自胆固醇酯水化的胆固醇相比，其摄取和分泌入胆汁的量很少[18]。 在体内，HDL 胆固醇酯在内涵体部份的水化可能在极性胆固醇的分泌中起着重要作用。 最近发现，SR -BⅠ 直接与胆固醇结合，提示选择性吸收的过程可能开始于细胞质膜，并且随着在胞内回吸收过程中胆固醇酯的水化而进一步进行。 在细胞内回吸收过程中,细胞表面的选择性吸收可能与细胞类型很有关系，例如，在生成类固醇激素细胞，选择性吸收可能发生在特定的胞质凹陷部分，而不涉及入胞作用[19] SR -BⅠ 的表达影响非 HDL 胆固醇的摄取 研究发现，

SR -BⅠ 不仅可以介导与脂蛋白相关的胆固醇的摄取，还可以介导游离胆固醇的摄取[20]。 因此，SR -BⅠ 的表达在胆固醇供需不同的组织很可能有不同的作用。 在不能利用胆固醇的外周组织(如动脉内皮细胞)，SR -BⅠ 的表达可以促进细胞中游离胆固醇向 HDL 的转移，而在肝、肾上腺、卵巢等的组织中,SR -BⅠ的表达则使 HDL 中的胆固醇酯转移至细胞内，即所谓选择性摄取胆固醇酯。 原位杂交实验发现，发生 AS 的 apoE 基因敲除小鼠变厚的主动脉内膜中，SR -BⅠ 的 mRNA 表达量增加[14]。 这表明 SR -BⅠ 可能促进胆固醇从发生 AS 的血管壁或泡沫细胞中流出，从而在预防 AS 中发挥作用。

在人类和某些动物模型上，血浆 HDL 胆固醇的水平和患动脉粥样硬化的危险性或严重程度成反比的关系[21]。 肝脏 SR -BⅠ表达可以增加逆向胆固醇的运输，从体内去除胆固醇。这样，SR -BⅠ是不是通过减少总的血浆 HDL 胆固醇而不利于动脉粥样硬化的形成? 在鼠身上答案是明确的，在 SR -BⅠ基因剔除的小鼠，可迅速加速动脉粥样硬化，而通过肝脏过度表达 SR -BⅠ 可抑制动脉粥样硬化，增加肝脏 SR -BⅠ 的表达有抗动脉粥样硬化效应[22]。这一结论为人们寻找用新的药物或基因治疗在工业化国家最常见的致死性疾病— — —心血管疾病提供了新的靶点。

参考文献:

[1] KriegerM. Charting the fate of the “good cholesterol” : identification and characterization of the high -density lipoprotein receptorSR-BI[J] .Annu Rev Biochem, 1999,68: 523 -558

[2] Silver DL. A Carboxyl, terminal PDZ-interacting domain of scavenger receptor B type I is essential for cell surface expression in liver[J] . J Biol Chem, 2002, 277(37) : 34042 -34047.[3] Kocher O, Yesilaltay A, Cirovic C, et al . Targeted disruption of the PDZK1 gene inmice causes tissue-specific depletion of the high density lipoprotein receptor scavenger receptor class B type I and altered li-poprotein metabolism[J] . Biol Chem,2003, 278(52) : 52820 -52825

[4] Acton S, Rigotti A , Landschulz KT, et al . Identification of scavenger re-ceptorSR -BI as a high density lipoprotein receptor [J] . Science, 1996,271 (5248) : 518 -520

[5] KriegerM. Charting the fate of the “good cholesterol” : identification and characterization of the high density lipoprotein receptor SR -BI [J] .Annu Rev Biochem,1999, 68: 523 -558.

[6] Ikemoto M, Arai H,Feng D, et al . Identification of a PDZ-domain -con -taining protein that interacts with the scavenger receptor class B type I [J] . Proc Natl Acad Sci USA , 2000, 97 (12) : 6538 -6543

[7] Connelly MA, De la Llera- Moya M, Peng Y, et al . Separation of lipid transport functions by mutations in the extracellular domain of scavenger receptor class Btype I[J] . J Biol Chem, 2003, 278(28) : 25773-25782.

[8] Uittenbogaard A, Shaul PW, Yuhanna IS, et al . High density lipoprotein prevents oxidized

low density lipoprotein prevents oxidized low density liploprotein -induced inhibition of endothelial nitric oxide synthase localization and activation in caveolae[J] . Biol Chem, 2000, 275(15) :11278 -11283
[9] Fazio S, Linton MF. Interplay between apolipoprotein E and scavenger receptor class B type Ⅰ controls coronary atherosclerosis and lifespan in the mouse[J] . Circulation, 2005, 111(25):3349- 3351.
[10] Zhang S, Picard MH, Vasile E, et al. Diet- induced occlusive coronary atherosclerosis, myocardial infarction, cardiac dysfunction, and premature death in scavenger receptor class B type I- deficient, hypomorphic apolipoprotein ER61 mice [J]. Circulation, 2005, 111(25):3457- 3464.
[11] Cao WM, Murao K, Imachi H, et al. Insulin- like growth factor- i regulation of hepatic scavenger receptor class BI [J]. Endocrinology, 2004, 145(12):5540- 5547.
[12] Svensson PA, Englund MC, Sn ckestrand MS, et al. Regulation and splicing of scavenger receptor class B type Ⅰ in human macropha-ges and atherosclerotic plaques [J]. BMC Cardiovasc Disord, 2005, 5:25.
[13] Webb NR, Connell PM, Graf GA, et al. SR -BⅡ, an isoform of the scavenger receptor BI containing an alternate cytoplasmic tail,mediates lipid transfer between high density lipoprotein and cells[J] . J BiolChem,1998, 273(24) : 15241 -15248
[14] Stangl H, Cao G , Wyne KL, et al. Scavenger receptor, class B, type I -dependent stimulation of cholesterol eserification by high density lipoproteins, low density lipoproteins, and nonlipoprotein cholesterol[J] .J Biol Chem,1998, 273(47) : 31002 -31008.
[15] Connelly MA, Williams DL. Scavenger receptor BI: a scavenger receptor with a mission to transport high density lipoprotein lipids [J]. Curr Opin Lipidol, 2004, 15(3):287- 295.
[16] Huby T, Doucet C, Dachet C, et al. Knockdown expression and hepatic deficiency reveal an atheroprotective role for SR- BI in liver and peripheral tissues [J] . J Clin Invest, 2006, 116(10):2767- 2776.
[17] Mineo C, Shaul PW. Functions of scavenger receptor class B, type Ⅰ in atherosclerosis [J]. Curr Opin Lipidol, 2012, 23(5):487- 493.
[18] Ji Y, Wang N , Ramakrishnan R, et al . Hepatic scavenger receptor BI promotes rapid clearance of high density lipoproteinfree cholesterol and its transport into bile. [J] . Biol Chem, 1999, 274(47) : 33398-33402.
[19] Reaven E, Leers -Sucheta S, Nomoto A , et al . Expression of scavenger receptor class B type I(SR-BI)promotes microvillar channel formation and selective cholesteryl ester transport in a heterologous reconstituted system[J] . Proc Natl Acad Sci, 2001, 98(4) : 1613 -1618
[20] Ueda Y, Royer L, Gong E, et al. Lower plasma levels and accelerated clearance of high density lipoprotein (HDL)and non -HDL cholesterol in scavenger receptor class B type I transgenic mice[J] . J Boil chem,1999, 274(11) : 7165-7171.
[21] Trigatti B,Covey S, Rizvi A. Scavenger receptor class B type I in high-density lipoprotein metabolism, atherosclerosis and heart disease : lessons from gene-targeted mice[J] . Biochem Soc Trans, 2004, 32(Pt 1) :116 -120.
[22] Ueda Y,Gong E, Royer L, et al . Relationship between expression levels and atherogenesis in scavenger receptor class B, type I transgenics[J] .Biol Chem, 2000, 275(27) : 20368 -20373.

Red同源重组技术在大肠杆菌糖基化基因敲除中的运用

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摘要：α-防御素-1（α-defensin-1，HNP-1）是人中性粒细胞的主要防御素分子，具有极强的抗菌和广泛的杀菌活性、细胞毒性及趋化作用，是当前国内外生物医学研究的热点，是机体天然防御体系的重要组成部分，研究者尝试通过基因工程技术使HNP-1高效表达。目前的研究集中在构建各种融合蛋白以降低对宿主的损害，从而提高HNP-1的表达效率，但是没有取得理想的效果。本课题组在原核诱导表达实验中首次发现在大肠杆菌中有成熟HNP-1的生成，并且成熟HNP-1也具有糖基化修饰。我们认为具有杀菌活性的成熟HNP-1的激活可能是导致宿主不能高效表达HNP-1的根本原因。由此我们拟采用Red同源重组技术敲除大肠杆菌宿主的糖基化基因，筛选并验证与HNP-1激活机制相关的蛋白。现就糖基化基因的敲除技术做一综述。

关键词：HNP-1　基因敲除　Red同源重组技术

0 引言

防御素是在中性粒细胞和上皮细胞中发现的一族富含半胱氨酸残基的阳离子短肽，人中性粒细胞中含有的防御素又名人中性粒细胞多肽，它们在中性粒细胞分化成熟前已合成，在生理情况下不能被诱导表达[1]。然而，人体中的防御素含量很少、结构相似，通过分离提纯获得生物活性较高的防御素单组分还存在一定的技术难度，化学合成又成本太高. 基因工程技术可以帮助人类获得特定的大量人类蛋白质，是获取目的蛋白的一个有效手段。

α -防御素-1（α -defensin-1，HNP-1）是人中性粒细胞的主要防御素分子，相对分子质量约3.5 $\times 10^3$ 道尔顿，主要存在于人中性粒细胞的溶酶体颗粒内，是迄今发现含量最多、活性最强的杀菌分子。HNP-1 对革兰氏阳性菌和革兰氏阴性菌以及厌氧菌、真菌、螺旋体、包膜病毒、腺病毒、流感病毒、艾滋病毒等均有很强的杀伤活性[2-5].。由于有活性的防御素对大肠杆菌有毒杀效应,所以传统的用大肠杆菌作为生物反应器直接表达有活性的防御素尚无成功报道. 有人把几个防御素基因连接起来共表达使之形成包含体但也同样需要解决没有活

性和首尾氨基酸与天然不同等问题,且包含体在后续的分离纯化及复性过程中要先进行溶解和洗涤。天然获取HNP-1 的量极其有限，而化学合成HNP-1 则由于其分子内含有3对二硫键，难以保证多肽折叠时它们的正确配对，从而影响其生物活性。因此，我们尝试通过基因工程的途径来获得HNP-1。HNP-1极有可能成为新一代抗菌药物，有助于解决细菌的耐药性和抗生素的毒副作用等问题，将HNP-1用于临床感染性疾病的防治是国内外医学界极感兴趣的课题。

1 HNP-1 的分子生物学特性

在HNP的生物合成过程中，首先翻译成包含一个信号序列的前原防御素（preproHNP-1)，后经逐步酶解和修饰形成成熟的HNP-1，最终转运并贮存于特定的细胞器嗜天青颗粒中。如在HNP-1的合成过程中, 首先翻译成94个氨基酸残基的preproHNP(包括19个氨基酸残基组成的疏水性信号序列,45个氨基酸残基组成的带负电荷的前区序列和C末端含30个氨基酸组成的带正电荷的成熟肽),进入内质网后，得到含56个氨基酸残基的原防御素(proHNP)，再在溶酶体中经一次或多次裂解、加工成为含30个氨基酸残基的阳离子成熟肽HNP-1[6-7]。在体内细胞合成HNP-1的过程中，由于先合成呈电中性的preproHNP，在翻译后的加工过程中封闭了防御素的细胞毒性，因而对细胞本身不引起损伤。HNP-1的抗菌活性受环境PH值、离子浓度、温度以及血清蛋白、成熟肽的ADP核糖化修饰等因素的影响。HNP-1还具有细胞毒、趋化作用、调理吞噬作用、参与补体激活等生物学活性。

2 HNP-1 的抗菌机理

目前认为HNP-1在体内的抗微生物的活性是通过对微生物表面膜的穿透，非特异地、迅速地杀灭微生物，即通过带正电荷的精氨酸与靶细胞静电吸附渗透靶细胞膜，疏水的HNP-1借助跨膜电位吸附到靶细胞膜上，然后HNP-1的单体或多聚体形成电压依赖膜通道，致使通常被隔离于细胞内外的分子(如电离子、多肽和蛋白质等)在无任何屏障的情况下无序扩散，进出靶细胞，尤其是胞内一些必要物质的泄漏，致使靶细胞死亡[8]。上述抗感染以及对恶性增生细胞的杀伤作用既是应用价值所在，也是基因工程中宿主不能高效表达HNP-1 的根本原因。在真核生物，如酵母、藻类中，研究者采用基因工程途径，通过融合表达的策略以改造

表达载体，但效果都不好。因此，在原核中，我们尝试通过改造大肠杆菌从而使HNP-1大量表达。

3 大肠杆菌中 HNP-1 糖基化现象的发现

在人体的HNP -1合成过程中还存在着翻译后修饰。研究发现中性粒细胞能表达精氨酸特异的ADP糖基化转移酶，并通过核糖基化转移酶(ART1)对富含精氨酸的HNP -1进行糖基化修饰。ADP核糖基化可以调控HNP-1的生物活性，在HNP -1成熟过程中具有重要作用[9-11]。我们从上述研究中获得启示：大肠杆菌可能也存在与人体相似的HNP-1激活机制。虽然是否确着存在这种机制还没有相关研究报道，但我们课题组前期试验数据初步证实了这种猜测，但不是本文所要讨论的主要内容，暂不赘述。

4 RecA 重组系统

既然猜测大肠杆菌中 HNP-1 的激活是由糖基化作用所致，而激活后的抗感染以及对恶性增生细胞的杀伤作用既是应用价值所在，也是基因工程中宿主不能高效表达 HNP-1 的根本原因（会杀死宿主细胞）。由此我们想到用基因敲除技术敲除大肠杆菌中的糖基化基因。在真核生物，如酵母、藻类中，研究者采用基因工程途径，通过融合表达的策略以改造表达载体，但效果都不好。因此，我们尝试通过改造大肠杆菌宿主从而使 HNP-1 大量表达。

RecA重组系统是大肠杆菌自身带有的非外源的同源重组系统，该系统主要由RecA蛋白和RecBCD复合蛋白组成。RecA蛋白在离体条件下，能促进同源DNA分子间的同源联会、配对、链交换和分支迁移，是重组过程中最重要的蛋白质[12]。RecA蛋白有两个主要特征：(1)单链DNA结合活性：RecA蛋白可以大量地同单链DNA结合，平均一个RecA单体能与4个核苷酸结合，此过程需要ATP；(2)依赖于DNA的ATP酶活性：在DNA存在时，RecA蛋白质能够水解ATP，从而促进链交换。RecBCD是由RecB、RecC和RecD组成的复合蛋白。RecBCD酶是一种多功能的酶，它具有依赖于ATP的单链和双链外切酶活性，因此又称为外切核酸酶V。由于RecBCD具有核酸外切酶V的活性，线性DNA分子在细菌体内会被降解，所以要利用微生物本身的RecA重组系统，就必需构建环状质粒打靶载体[13]，且过程繁琐，重组效率不高。

5 Red同源重组系统

虽然对传统的同源重组技术做了很多改进，RecA介导的同源重组还是有很大的不足：首先是需要较长的目的基因同源臂，其次由于RecBCD的核酸外切酶活性所以需要构建环状质粒，操作繁琐，增加了工作量和实验成本，另外重组效率太低，要想获得重组子需对大量样本进行筛选，费事费力。线性DNA介导的同源重组是近十几年来研究的热点之一。它利用大概50bp左右的同源臂，可以实现基因的插入、缺失、置换、对基因片段的精确修改、基因克隆等。与传统的依赖特定的酶切位点的方法相比，这种方法避免了酶切、酶连、质粒构建等基因操作，因而方便、精确、节省时间和成本。直接将线性DNA片段，通常是PCR产物或者合成的线性单链DNA作为打靶片段，电转到目的细胞，诱导催化重组过程的蛋白表达，即可实现重组过程。

噬菌体的Red重组系统由gam、bet、exo三个基因组成。gam基因产物为Gam蛋白，分子量16KD，它能够抑制大肠杆菌内的RecBCD蛋白复合体的核酸外切酶活性，防止线性DNA片段被降解[14]。bet基因的产物Beta蛋白是一个单链结合蛋白，分子量28KD，它可以和单链结合促进互补链的退火形成新的双链分子[15-17]。exo基因的产物为Exo蛋白，该蛋白有5’-3’方向的核酸外切酶活性，可以从5’端开始降解双链DNA，从而产生一个3’的突出末端[18]。要利用Red同源重组系统实现高效的同源重组，Red重组酶的高效表达就很重要。Gam蛋白的限制性表达使得Gam蛋白的毒性副作用和重复序列间的重组减少到最小程度[19]。

由于Red同源重组系统的高效性，利用PCR扩增产物或人工合成的线性片段电转到宿主菌中，在重组酶的作用即可实现同源重组，一般使用35～50 bp同源臂即可。这种技术省去了体外DNA酶切、酶连等步骤，逐渐在分子生物学实验中广泛应用。从1998年Red同源重组技术开始研究到现在，有很多研究者利用这个技术对大肠杆菌染色体基因组进行了靶向修饰。Wanner课题组研究者用PCR方法合成线性打靶片段，PCR引物包含两部分，5’端有36---50个碱基与靶基因两端同源，3’端与PCR反应的模板序列两端互补。用这样的引物进行PCR后即得到两侧带有36--50个同源臂，中间是筛选标记基因的线性打靶片段，能够对染色体上需要进行基因修饰的区域快速且准确的定位，用这种方法他们成功地敲除了大肠杆

菌的13个基因[20]。对于体内操作，可以先把质粒DNA转化到宿主菌中后再电转线性打靶片段，也可以在电转线性打靶片段的时候一起电转质粒DNA。

具体到我所在课题组，我们的实验思路是：采用Red 重组技术，利用λ 噬菌体Red重组酶将导入细胞的线性DNA片段与染色体的特定靶基因序列进行同源重组。首先构建基因打靶线性片段（两端为目的基因同源序列，中间为抗性基因筛选标记），然后将能够表达Red 重组酶的质粒转入到宿主菌中，并将其制备成感受态，随后将目的基因打靶片段转化到受体细胞中，并进行筛选，获得需要的重组体，最后将抗性筛选标记去除，得到敲除目的基因的大肠杆菌。

6 存在的问题

虽然 Red 同源重组技术是近年来研究比较火热的技术，但落实到具体实验还是有很多细节问题需要探索。首先，采用 Red 同源重组技术进行基因敲除从本质上讲是个概率问题，只是会相对增加其打靶敲除成功的概率。Red 基因的诱导表达和电转化感受态细胞制备过程以及细菌的浓度状态等都将影响重组的最终效率。电转的成功率不高也是实验中遇到的困难之一。具体到不同的质粒，不同的宿主菌，实验条件及方法都有不同，单从现有文献报道难以获取，且温度对整个实验的成功与否有很大的影响。这些都需要在具体的实验过程中自己尝试，自己解决。

7 期望

如果能够掌握好这个技术，成功敲除大肠杆菌基因组中的一个基因，那结果会是可喜的。试想，大肠杆菌中表达的 HNP-1 是受到了某种翻译后修饰（目前预测为糖基化修饰），正式由于这种修饰激活，成熟的 HNP-1 有了杀菌能力，如果我们筛选出与修饰有关的基因(不断筛选中，可用此技术敲除多个不同的基因)，即可对此基因进行敲除。至少会发现这些基因的功能，获得与 HNP-1 激活相关的数据。再将 HNP -1 克隆到 pET-28a(+)载体上，转入到大肠杆菌中诱导表达，此时宿主菌就可大量表达 HNP-1 前体蛋白，再经过提取纯化，大量获得它就有很大希望，我对此也是充满信心。

参考文献

[1] Ganz T，Weiss J. Antimicrobial peptides of phagocytes and epithelia[J]. Hematol ，1997；34：343 - 354.

[2] Selsted ME, Ouellette AJ. Mammalian defensins in the antimicrobial immune response. *Nature immunology.* Jun 2005;6(6):551-557.

[3] Lehrer RI, Lu W. alpha-Defensins in human innate immunity. *Immunological reviews.* Jan 2012;245(1):84-112.

[4] Tenge VR, Gounder AP, Wiens ME, Lu W, Smith JG. Delineation of interfaces on human alpha-defensins critical for human adenovirus and human papillomavirus inhibition. *PLoS pathogens.* Sep 2014;10(9):e1004360.

[5] Chang TL, Vargas J, Jr., DelPortillo A, Klotman ME. Dual role of alpha-defensin -1 in anti-HIV-1 innate immunity. *The Journal of clinical investigation.* Mar 2005 ; 115(3):765-773.

[6]Valore EV, Ganz T. Posttranslational processing of defensins in immature human myeloid cells. *Blood.* Mar 15 1992;79(6):1538-1544.

[7] Wu Z, Prahl A, Powell R, Ericksen B, Lubkowski J, Lu W. From pro defensins to defensins: synthesis and characterization of human neutrophil pro alpha-defensin-1 and its mature domain. *The journal of peptide research : official journal of the American Peptide Society.* Aug 2003;62(2):53-62.

[8]Hilchie AL, Wuerth K, Hancock RE. Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. *Nature chemical biology.* Dec 2013 ; 9(12): 761-768.

[9]Paone G, Wada A, Stevens LA, Matin A, Hirayama T, Levine RL, Moss J. ADP ribosylation of human neutrophil peptide-1 regulates its biological properties. *Proceedings of the National Academy of Sciences of the United States of America.* Jun 11 2002;99(12):8231-8235.

[10] Paone G, Stevens LA, Levine RL, Bourgeois C, Steagall WK, Gochuico BR, Moss J. ADP-ribosyltransferase-specific modification of human neutrophil peptide-1. *The Journal of biological chemistry.* Jun 23 2006;281(25):17054-17060.

[11]Stevens LA, Barbieri JT, Piszczek G, Otuonye AN, Levine RL, Zheng G, Moss J. Nonenzymatic Conversion of ADP-Ribosylated Arginines to Ornithine Alters the

Biological Activities of Human Neutrophil Peptide-1. *Journal of immunology.* Dec 152014;193(12):6144-6151.

[12]Roca AI，Cox M M．RecA protein：structure，function，and role in recombinational DNA repair．Prog Nucleic Acid Res Mol Bi01．1 997，56：1 29—223．

[13]Dueger E L，House J K，HeithoffD M，et a1．Salmonella DNAadenine methylase mutants elicit protective immune responses to homologous and heterologous serovars in chickens． Infect Immun．200 1，69(1 2)：7950-7954．

[14]Murphy K C．Lambda Gamprotein inhibits the helicase and chi．stimulated recombination activities of Escherichia coli RecBCD enzyme．J Bacteri01 1991． 173(18)：5808．5821．

[1 5]Kuzminov A．Recombinational repair of DNA damage in Escherichia coli and bacteriophage lambda．Microbiol Mol Biol Rev．1999．63(4)：751．813．

[1 6]Poteete A R，Fenton A C．Genetic requirements of phage lambda red．mediated gene replacement in Eschefichia coli K．12．J Bacteri01．2000．1 82(8)：2336．2340．

[1 7]Li Z，Karakousis G Chiu S K，et a1．The beta protein of phage lambda promotes strand exchange．J Mol Bi01．1 998．276(4)：733-744．

[18]Little J W，Lehman I R，Kaiser AD．An exonuclease induced by bacteriophage lambda．I Preparation of the crystalline enzyme．J Biol Chem．1 967．242(4)：672—678．

[19]Sergueev K，Yu D，Austin S，et a1．Cell toxicity caused by products of the p(L)operon of bacteriophage lambda．Gene．2001，272(1-2)：227—235．

[20]Datsenko K A，Wanner B L．One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products．Proc Natl Acad Sci U S A．2000，97(12)：6640-6645．