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NKX 蛋白结构和功能的研究进展

王丹彤, 陈 成*
(天津大学 生命科学学院, 中国天津 300072)

摘 要: NKX 蛋白是 NKL 同源框转录因子亚类中最主要的类型, 被发现广泛参与调控胚胎发育过程中的细胞命运决定与形体模式形成, 尤其对神经系统的发育影响甚广, 并参与甲状腺癌、肺癌、前列腺癌等多种肿瘤的发生。鉴于 NKX 蛋白在胚胎发育和肿瘤发生过程中的重要作用, 本文系统性综述了 NKX 同源框蛋白 7 个家族 17 个蛋白质的结构生物学、生理机制以及疾病相关性的研究进展, 为相关疾病的基因靶向治疗提供理论基础, 同时讨论了一些 *NKX* 基因缺乏组学研究和模式动物疾病模型构建等问题, 为今后的研究提供方向。

关键词: NKX 同源框蛋白; 三维结构; 生理机制; 病理相关性
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Progress in Structure and Function of NKX Proteins

WANG Dantong, CHEN Cheng*
(*College of Life Sciences*, *Tianjin University*, *Tianjin* 300072, *China*)

Abstract: NKX proteins are the most important type of NKL homeobox transcription factor subclass and have been found to be widely involved in regulating cell fate and body pattern formation during embryonic development, especially embryonic development of nervous system, and involved in the occurrence of thyroid cancer, lung cancer, prostate cancer and other tumors. In view of the important roles of NKX proteins in embryonic development and tumorigenesis, the research progress of the structural properties, physiological mechanisms, and disease correlation of 17 proteins in the 7 NKX homeobox protein families were systematically summarized, which would provide helpful information on gene targeted therapy of related diseases. In addition, the lack of genomics research and animal model construction of diseases related to some *NKX* genes were also discussed, which should be directions for future research.

Key words: NKX homeobox protein; three-dimensional structure; physiological mechanism; pathological correlation

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1984 年, McGinnis 等[1]首次在黑腹果蝇(*Drosophila melanogaster*)的同源异型基因中发现了一段 180 bp 的保守 DNA 序列, 称之为同源框(homeobox), 含有同源框的基因统称为同源框基因(homeobox gene)。此后不久, 研究人员在无脊椎动物[2]、脊椎动物[3]和人类[4]中也发现了同源框基因。同源框基因编码一个含 60 个氨基酸的 DNA 结合基序, 该基序被称为同源结构域(homeodomain, HD), 包括 3 个 α-螺旋和 N-末端延伸, 其中 α-螺旋Ⅰ和Ⅱ相互平行, α-螺旋Ⅲ与 DNA 的大沟槽结合, N-末端与相邻小沟槽结合, 含有同源结构域的蛋白质被称为同源框蛋白(homeobox protein)[5]。在动物进化过程中, 同源框基因分化为 12 个基因类型, 其中 *ANTP* (*antennapedia*)和 *PRD* (*paired*)为主要类型, *ANTP* 类同源框基因又分为 *HOXL* (*homeobox-like*)和 *NKL* (*NK-like*)两个亚类[6](表1)。1989 年, Nirenberg 和 Kim[7]首次报告了果蝇中的 *NK* 基因, 其与随后鉴定出的很多同源基因一起构成了 *NKL* 同源框基因亚类。其中, *NKX* (*NK homeobox*)基因在人类 *NKL* 亚类中占 35%, 为该亚

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作者简介: 王丹彤(1997—), 女, 河北保定人, 硕士研究生; * 通信作者: 陈成(1985—), 男, 江苏连云港人, 博士, 副教授, 主要从事结构生物学研究, E-mail: chengchen@tju.edu.cn。

类中最主要的基因类型, 因此近年来得到广泛研究[8-11]。目前, 研究人员已从人类同源框基因中发现 17 个 *NKX* 基因, 并根据同源性将它们分为 7 个基因家族[12](表 2)。

研究发现, NKX 蛋白广泛参与调控胚胎发育过程中的细胞命运决定与形体模式形成, 并与多种肿瘤的发生相关[8-11]。本文通过回顾历年来的研究成果, 对 NKX 同源框蛋白的结构生物学以及生理机制、病理相关性的研究进展进行综述, 旨在为今后相关疾病的基因靶向治疗提供理论基础。

1 NKX 蛋白的结构研究进展

NKX 蛋白作为转录因子, 在胚胎发育和肿瘤发生过程中具有关键调控作用, 其突变或缺失会导致神经系统、心脏、肠、胃、胰腺、肺等多个部位疾病的发生。研究人员在其生化和结构方面开展研究, 以求从分子层面认识其与核酸底物的精细识别模式, 从而阐明其病变机理。到目前为止, 已有 NKX2-1、NKX3-1 和 NKX2-5 三个 NKX 蛋白的三维结构被报道。

NKX2-1[13]和 NKX3-1[14]的同源结构域与DNA复合物的高分辨率结构如图 1 所示。

NKX2-1 蛋白的结构包括同源结构域和两个转录激活域(transcription activating domain, TAD); 转录激活域一个位于 N 端, 另一个位于 C 端, 两者都可以与 DNA 结合, 其中 N 端转录激活域 TAD1 参与调节甲状腺和肺特异性基因的转录[13](图1A)。电泳迁移率变动分析(electrophoretic mobility shift

表 1 人类同源框基因的分类(参照文献[12]绘制)

Table 1 Classification of human homeobox genes (created according to Reference [12])

Class	Subclass	Number of gene family	Number of gene	Number of pseudogene
ANTP	*HOXL*	14	52	0
	NKL	23	48	19
PRD		3	7	0
PRD-LIKE		28	43	24
LIM		6	12	0
ZF		5	14	1
POU		7	16	8
HNF		2	3	0
CUT		3	7	3
PROS		1	2	0
CERS		1	5	0
SIX/SO		3	6	0
TALE		6	20	10
Total		102	235	65

表 2 人类 *NKX* 同源框基因(参照文献[12]绘制)

Table 2 Human *NKX* homeobox genes (created according to Reference [12])

Family	Gene name	Location	Gene ID	Other name
*NK*1	*NKX*1-1	4p16.3	54729	*HSPX*153, *HPX*153
	*NKX*1-2	10q26.13	390010	*C*10*orf*121
*NK*2.1	*NKX*2-1	14q13.3	7080	*NKX*2*A*, *TTF*1, *TITF*1
	*NKX*2-4	20p11.22	644524	*NKX*2*D*
*NK*2.2	*NKX*2-2	20p11.22	4821	*NKX*2*B*
	*NKX*2-8	14q13.3	26257	*NKX*2*H*
*NK*3	*NKX*3-1	8p21.2	4824	*NKX*3*A*
	*NKX*3-2	4p15.33	579	*NKX*3*B*, *BAPX*1
*NK*4	*NKX*2-3	10q24.2	159296	*NKX*2*C*, *NKX*4-3, *CSX*3
	*NKX*2-5	5q35.1	1482	*NKX*2*E*, *NKX*4-1, *CSX*, *CSX*1
	*NKX*2-6	8p21.2	137814	*NKX*4-2, *CSX*2
*NK*5	*HMX*1	4p16.1	3166	*NKX*5-3, *H*6
	*HMX*2	10q26.13	3167	*NKX*5-2, *H*6*L*
	*HMX*3	10q26.13	340784	*NKX*5-1
*NK*6	*NKX*6-1	4q21.23	4825	*NKX*6*A*
	*NKX*6-2	10q26.3	84504	*NKX*6*B*, *GTX*
	*NKX*6-3	8p11.21	157848	/

assay, EMSA)实验结果证明, NKX2–1 的 α–螺旋(α–helix) Ⅲ可以识别特异性的 DNA 序列 5′–CAAG–3′, 而不是更常见的 5′–TAAT–3′序列, 这种特异性主要由同源结构域氨基酸残基 Q50 和 Y54 决定[13]。由于 NKX2–1 和 DNA 复合物结构中的 DNA 不是内源性基因片段, 不能解释其在体内的互作模式, 因此其致病机制的研究受限。

NKX3–1 特异表达于前列腺管腔上皮细胞, 是一种肿瘤抑制蛋白, 3 种错义突变体 p.A17T、p.R52C 和 p.T164A 影响前列腺上皮细胞的生长与分化, 可能与前列腺癌的发生相关[14], 其中 p.T164A 突变位于 NKX3–1 蛋白的同源框结构域(对应同源结构域的 41 位)。目前, 研究人员只得到了 NKX3–1 蛋白同源结构域的单体结构[14], 如图 1B 所示, T41 与 α–螺旋Ⅲ第三残基 Q44 的主链酰胺形成氢键, Q44 侧链的羰基氧也可以与 T41 的主链酰胺形成氢键, 这种 N_{cap}→N_3 氢键网络有助于稳定同源结构域的 α–螺旋Ⅲ, 而前列腺癌 NKX3–1^{T164A} 突变体的丙氨酸残基缺少羟基侧链, 这会导致其中一个 N_{cap} 氢键无法形成, 从而影响同源结构域的稳定性以及与 DNA 的识别和结合[14]。当前, NKX3–1 与 DNA 复合物的结构还未被解析, 二者的结合模式尚不能得到很好地解释, 因此 NKX3–1 与 DNA 复合物三维结构信息的获取可作为今后的研究内容。

NKX2–5 同源结构域和 DNA 复合物的高分辨率结构[15]如图 2A 所示。整体结构中两个 NKX2–5 同源结构域占据一个 DNA 的两个结合位点, 该 DNA 为内源性靶点(心钠素基因 *ANF* 的近端启动子 242 位点), 包括由 5 个碱基对隔开的两个结合基序(TGAAGTG/TCAAGAG)。图 2B 显示了与心脏病形成相关的 NKX2–5 同源结构域的错义突变位点, EMSA 和动力学模拟的研究结果表明, 与DNA 直接接触的突变 p.R5C、p.Q50H、p.N51K、p.R53H、p.R53C 和 p.Y54C 可能会影响 DNA 的结合, 而不直接接触 DNA 的氨基酸残基突变 p.L34P、p.T41M、p.W47L 和 p.R52G 会影响蛋白质结构的稳定性, 因此这些突变都可能会影响 NKX2–5 作为转录因子的调控功能[15]。

2　NKX 蛋白的生理机制和疾病相关性

NKX 蛋白对胚胎发育和肿瘤发生过程起着重要的调控作用, 并且具有多种疾病相关性(表3)。一直以来, 研究人员致力于 NKX 蛋白生理机制和致病机理的研究, 以求为相关疾病的基因靶向治疗提供理论基础。

2.1　NKX 蛋白在胚胎发育过程中的作用

NK1 同源框蛋白家族成员有 NKX1–1 和NKX1–2。NKX1–1 在眼外肌、后连合束的中脑神经元、后脑的腹侧神经元和脊髓的中间神经元中都有表达, 能通过影响涡虫纤维的形成在体壁肌中提供身体模式形成所需的位置信息[16], 但是 NKX1–1 参与调控的具体分子机制目前尚不明确。NKX1–2 在发育早期的神经中胚层祖细胞中表达, 参与 Wnt/β–catenin 信号通路下游基因调控网络, 调控中、内胚层的形成, 具体机制如图 3 所示, NK–

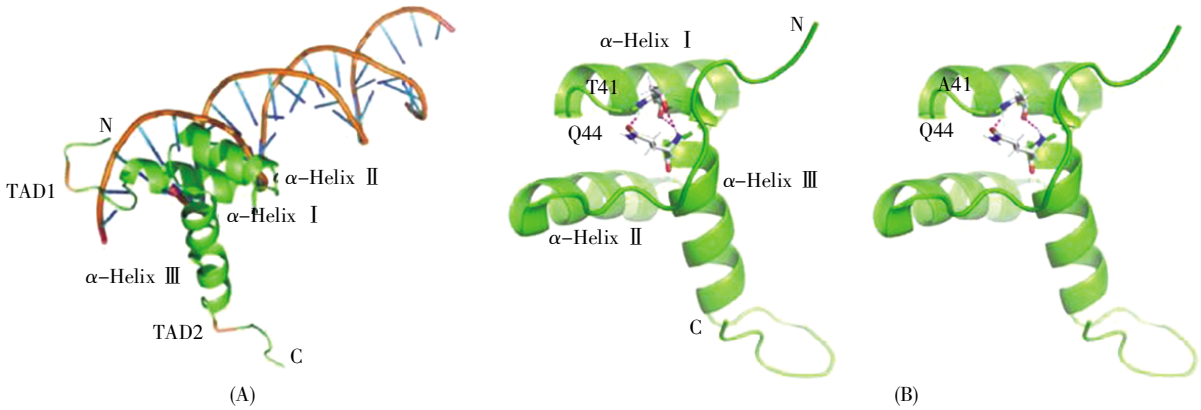

图 1　NKX2–1 和 NKX3–1 同源结构域的三维结构(参照文献[13]和[14], 使用 PyMOL 绘制)

(A) NKX2–1 同源结构域与 DNA 复合物的结构; (B) 野生型 NKX3–1 (左)和突变体 NKX3–1^{T164A} (右)同源结构域的结构。灰色、蓝色和红色分别表示 C 原子、N 原子和 O 原子; 粉色虚线表示氢键。

Fig.1　The three–dimensional structures of NKX2–1 and NKX3–1 HDs (created with PyMOL, according to References [13] and [14])

(A) The structure of NKX2–1 HD and DNA complex; (B) The HD structures of wild–type NKX3–1 (left) and NKX3–1^{T164A} mutant (right). The colors gray, blue and red represent C, N, and O atoms, respectively, and the pink dotted lines represent hydrogen bonds.

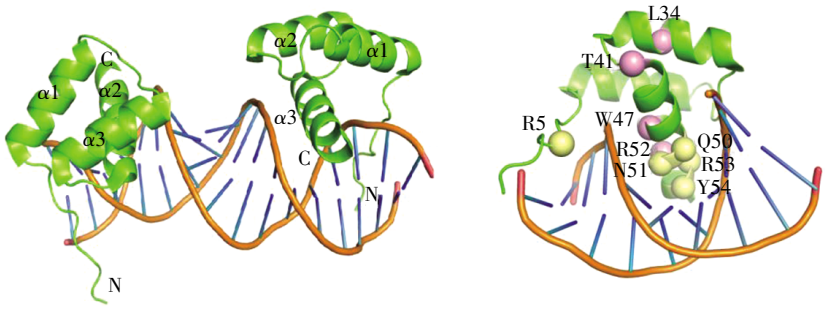

图 2 NKX2–5 同源结构域与 DNA 复合物的三维结构(参照文献[15], 使用 PyMOL 绘制)

(A) NKX2–5 同源结构域和 ANF–242 复合物的整体结构; (B) 心脏病相关的 NKX2–5 同源结构域的突变位点。黄色球体表示与 DNA 直接接触的位点; 粉色球体表示不与 DNA 直接接触的位点。

Fig.2 The three–dimensional structure of NKX2–5 HD and DNA complex (created with PyMOL, according to Reference [15])

(A) The overall structure of NKX2–5 HD and ANF–242 complex; (B) Mutations in NKX2–5 HD associated with heart disease. The yellow and pink spheres represent sites having direct and non–direct contact with DNA, respectively.

X1–2 的转录被 Wnt/β–catenin 信号通路激活, 激活的 NKX1–2 通过下调转录因子 3 (transcription factor 3, TCF3)和其他潜在抑制靶点的表达来激活 *Brachyury* (T–box 转录因子)基因的转录, 相反, Brachyury 通过激活某介体抑制 NKX1–2 的表达, 因此 Wnt/β–catenin 信号通路有效激活 Brachyury 的表达需要 NKX1–2 的上调[17](图 3)。NKX1–2 还可以通过抑制抗脂肪生成的鸡卵清蛋白上游启动子转录因子Ⅱ (chicken ovalbumin upstream promoter–transcription factor Ⅱ, COUP–TF Ⅱ)的表达促进脂肪生成, 并可能在调节 ST2 骨髓间充质前体细胞的脂肪细胞和成骨细胞分化之间的平衡中发挥作用[18]。

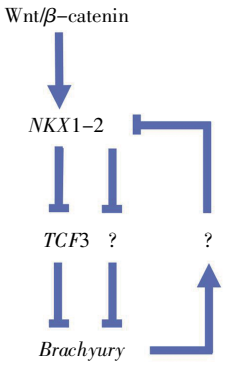

图 3 *NKX*1–2 参与的中、内胚层形成的基因调控网络(参照文献[17], 使用 Adobe Illustrator 绘制)

Fig.3 The gene regulatory network involving *NKX*1–2 in mesoderm and endoderm formation (created with Adobe Illustrator, according to Reference [17])

NK2.1 同源框蛋白家族成员有 NKX2–1 和 NKX2–4。NKX2–1 主要表达于前肠内胚层和神经外胚层分化的细胞, 在肺的胚胎发育过程中, NKX2–1 可调控肺形态发生、肺上皮细胞分化、肺表面活性物质蛋白(SP–A、SP–B 和 SP–C)和支气管上皮细胞 Clara 细胞分泌蛋白的转录; 在甲状腺的胚胎发育过程中, NKX2–1 和其他甲状腺转录因子(PAX8、FOXE1 和 HHEX)在甲状腺前体细胞和甲状腺滤泡细胞中共同表达, 且在成熟甲状腺细胞中持续表达, 从而建立并维持甲状腺的表型、功能、内环境平衡和组织分化[19]。相关研究报道, NKX2–1 的若干临床突变(p.V45F、p.E175Ter、c.2595insGG、c.255insG、c.376–2A–G)会导致先天性甲状腺功能减退伴肺功能障碍[20]。除了甲状腺和肺, NKX2–1 也在垂体、下丘脑和其他间脑组织中表达, 并参与脑组织的发育和生物功能[9], 例如: 其通过调节胶质纤维酸性蛋白(glial fibrillary acidic protein, GFAP)的表达影响星形胶质细胞的生成[21]。NKX2–4 与 NKX2–1 在下丘脑发育中有部分冗余作用, NKX2–1 控制下丘脑头腹侧发育, 包括苍白球和视前区, 而 NKX2–4 控制下丘脑吻部、中部和尾部的发育[22]。除此之外, NKX2–4 和另一 NKX 蛋白 NKX2–3 的异常表达会导致特定的急性髓系白血病(acute myeloid leukemia, AML)亚型的形成, 具体机制如图 4 所示。已知 FLI1 (friend leukemia integration 1)在抑制红细胞分化的同时可激活巨核细胞分化, 在 AML 细胞系 OCI–M2 中, 内皮发育相关转录因子 SOX7 [sex determining region Y (SRY)–related high–mobility group (HMG) box transcription factor 7]、干扰素调节因子 6 (interferon regulatory factor 6, IRF6)、HEY1 (hairy/enhancer–of–split related with YRPW motif protein 1)和 ETS 变体 2 [E26 transformation–specific (ETS)

variant 2, ETV2]异常激活 NKX2–4 的表达, 从而下调 FLI1 的转录水平, 不同的是, 在 AML 细胞系 ELF–153 中 NKX2–3 会异常激活 FLI1 的转录, 但是结果均导致巨核细胞和红细胞分化异常[10]。

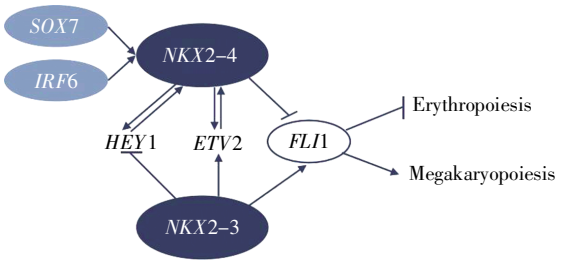

图 4　*NKX2–4* 和 *NKX2–3* 参与的 AML 形成的基因调控网络(参照文献[10], 使用 Adobe Illustrator 绘制)

Fig.4　The gene regulatory network involving *NKX2–4* and *NKX2–3* in AML formation (created with Adobe Illustrator, according to Reference [10])

NK2.2 同源框蛋白家族成员有 NKX2–2 和 NKX2–8。NKX2–2 参与胚胎神经管 V3 域的模式形成, 调控少突胶质细胞后期的成熟与发育[23], 并参与决定后脑和内脏运动神经元的细胞命运[24]。除神经细胞外, NKX2–2 还参与决定胰岛祖细胞命运。NKX2–2 能够使特定的胰岛 β 细胞基因表达, 而 NKX2–2 缺失会使小鼠 β 细胞祖细胞转变为产生胃饥饿素的细胞, 导致严重的糖尿病与极低出生体重儿、儿童肥胖和发育迟缓[8]。NKX2–8 在胚胎发育时期表达于内胚层, 参与早期心脏和肺部发育[25]。

NK3 同源框蛋白家族成员有 NKX3–1 和 NKX3–2。NKX3–1 是第一个已知的前列腺上皮特异性标记物, 是一种参与睾丸和前列腺发育的转录因子, 通过抑制雄激素受体(androgen receptor, AR)的表达, 激活磷脂酰肌醇 3 激酶/蛋白激酶 B (phosphatidylinositol 3–kinase/protein kinase B, PI3K/Akt)信号通路, 参与前列腺上皮细胞命运决定和维持正常的前列腺分泌功能[26]。NKX3–2 与骨骼发育相关, 其不仅能够通过激活软骨分化相关蛋白质成纤维细胞生长因子受体 3 (fibroblast growth factor receptor 3, FGFR3)、成骨细胞特异因子 2 (osteoblast–specific factor 2, OSF2)、印度刺猬因子(Indian Hedgehog, IHH)、SOX9 的转录促进软骨的发育, 而且可通过激活细胞外基质蛋白质 X 型胶原 α1 [collagen alpha 1 (X) chain, COL10A1]、Ⅱ型胶原 α1 [collagen alpha 1 (Ⅱ) chain, COL2A1]、Ⅸ型胶原 α2 [collagen alpha 2 (Ⅸ) chain, COL9A2]、蛋白聚糖(proteoglycan, PG)、骨桥蛋白(osteopontin, OPN)的转录调控脊椎椎间盘和锥体的分化过程, NKX3–2 的缺失会导致软骨细胞凋亡[27]。Simsek–Kiper 等[28]发现, NKX3–2^{Gly171Cysfs*55} 突变会导致脊椎骨干骺端发育不良。此外, NKX3–2 还参与内脏中胚层的模式形成, 同时影响胃间质和脾脏的发育[29]。

NK4 同源框蛋白家族成员有 NKX2–3、NKX2–5 和 NKX2–6。NKX2–3 在微血管内皮细胞和胃肠道肌层黏膜中表达。NKX2–3 通过激活 AMP 活化蛋白激酶/哺乳动物雷帕霉素靶蛋白(AMP–activated protein kinase/mammalian target of rapamycin, AMPK/mTOR)信号通路抑制平滑肌细胞的增殖和迁移, 从而抑制内膜增生诱导的血管再狭窄形成[30]。同时, *NKX2–3* 基因表达水平的降低会抑制内皮素 1 (endothelin 1, EDN1)和血管内皮生长因子(vascular endothelial growth factor, VEGF)信号通路以及 PI3K/Akt–内皮型一氧化氮合酶(endothelial nitric oxide synthase, eNOS)信号通路, 从而导致炎症性肠病的产生[31]。不仅如此, NKX2–3 还参与外周淋巴器官的发育和分化[32]。NKX2–5和 NKX2–6 主要参与心脏的形态发生, 其突变会导致一系列心脏相关疾病。比如: NKX2–5^{V182M}、NKX2–5^{K183X}、NKX2–6^{V176M} 和 NKX2–6^{K177X} 突变体会导致其转录激活功能明显减弱, 从而引起法洛四联症或右心室双出口和室间隔缺损[33]; NKX2–5^{K158Q} 和 NKX2–6^{K152Q} 突变体会导致其转录激活功能明显减弱, 进而引起室间隔缺损[34]; NKX2–5^{Q181H} 与 NKX2–6^{Q175H} 突变体会使心房利钠因子的转录活性显著降低, 进而引起心房颤动[35]。因此, 针对 NKX2–5 和 NKX2–6 的研究有助于开发心脏病预防和心脏保护治疗策略。除心脏外, NKX2–5 和 NKX2–6 在胚胎发育早期咽的腹侧区域也有表达, 并参与调节咽部内胚层细胞的分化、增殖和生存[36]。近期又有研究表明, NKX2–5 能够调控碘有机化、甲状腺球蛋白合成、碘化物运输和碘化酪氨酸脱碘相关基因的表达, 从而影响甲状腺分化和发育, NKX2–5^{A119S} 突变会导致先天性原发性甲状腺功能减退症[37]。

NK5 同源框蛋白家族成员有 HMX1 (NKX5–3)、HMX2 (NKX5–2)和 HMX3 (NKX5–1)。HMX1 广泛表达于眼睛、周围神经节和鳃弓, 可调节颅面发育, 抑制 HMX1 的表达会导致视网膜祖细胞细胞周期异常, 视网膜分化迟缓, 进而形成小眼症[38]。2015 年, Gillespie 等[39]鉴定了突变体 HMX1^{Q217P} 与

眼耳综合征的发生相关。HMX2 与 HMX3 在耳上皮细胞中共表达, 通过激活发育调节因子骨形态发生蛋白 4 (bone morphogenetic protein 4, BMP4)、远端缺失同源框蛋白 5 (distal-less homeobox 5, DLX5)、成对盒蛋白(paired box protein, PAX) 2 和 5 的转录影响内耳发育, HMX2 缺失会导致前庭功能受损[40]。此外, HMX2 和 HMX3 可调控骨髓造血干细胞分化, 参与了 AML 形成的基因调控网络, 如图 5 所示, 白细胞介素-7 (interleukin-7, IL-7)、干扰素调节因子 IRF8、转录因子 ETS1、ETS 结构域包含蛋白 1 (ETS domain-containing protein 1, ELK1)和转录因子 SP1 (specificity protein 1)激活 HMX2/3 的表达, 肿瘤坏死因子-α/核因子-κB (tumor necrosis factor-α/nuclear factor-κB, TNF-α/NF-κB)信号抑制 HMX2/3 的表达, 在 AML 细胞系中, IL-7 受体的突变、IRF8 的过表达和特异性 Wnt 信号都会异常激活 HMX2/3 的表达, 同时, HMX2/3 的两个转录因子结合位点突变可分别导致新的 ETS 结合位点的产生和 NF-κB 结合位点转化成 SP1 结合位点, 这两种突变也都可促进 HMX2/3 的转录激活, 而 HMX2/3 抑制细胞分化基因 *EPX* 的表达, 同时激活 5-羟色胺受体 7 (5-hydro-xytryptamine receptor 7, HTR7)的表达, 进而增强致癌的胞外信号调节激酶(extracellular signal-regulated kinase, ERK)信号[41]。

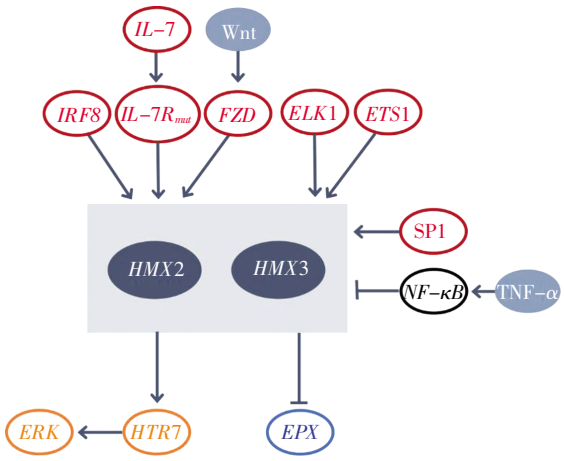

图 5 *HMX2* 和 *HMX3* 参与的 AML 形成的基因调控网络(参照文献[41], 使用 Adobe Illustrator 绘制)

Fig.5 The gene regulatory network involving *HMX2* and *HMX3* in AML formation (created with Adobe Illustrator, according to Reference [41])

NK6 同源框蛋白家族成员有 NKX6-1、NK-X6-2 和 NKX6-3。NKX6-1 参与 SHH 信号控制的级联反应, 调控中脑多巴胺能神经元的发育, 影响大脑的正常功能并参与严重的神经疾病(如帕金森病)的形成[42]。除与神经系统发育相关外, NK-X6-1 还在胰腺祖细胞中表达, 为激活胰腺祖细胞分化为单激素 β 细胞所必需, 其缺乏会使 β 细胞脱分化或转分化以及功能丧失, 从而导致 2 型糖尿病的形成[43]。不仅是 β 细胞, NKX6-1 还可以通过调控胰腺 α 细胞中 ARX (aristaless-related homeobox)的表达来影响 α 细胞的数量和胰高血糖素的分泌[44]。NKX6-2 主要在发育中的中枢神经系统表达, 参与脊髓神经元的发育和脊髓运动回路的形成[11], 调控端脑、中脑和间脑基底核神经元祖细胞的确定和分化[45], 并通过调控外周髓鞘蛋白22 (peripheral myelin protein 22, PMP22)、SOX10 和酪氨酸- tRNA 连接酶(tyrosine-tRNA ligase, YARS)等的表达影响少突胶质细胞成熟及髓鞘发育[46], 目前的研究已经发现, NKX6-2 的 15 种突变(p.L163V、p.L181V、p.N198D 等)会导致一系列由中枢神经系统脱髓鞘引起的进行性神经表现, 如共济失调症[47]。此外, NKX6-2 与 NKX6-1 一样, 在胰腺祖细胞中也具有 α 细胞和 β 细胞命运决定的作用[48]。NKX6-3 主要在发育中的中枢神经系统[49]和胃肠道[50]表达。在神经胚期, NKX6-3 激活 Wnt 和成纤维细胞生长因子(fibroblast growth factor, FGF)信号通路, 抑制 BMP 信号通路, 从而促进神经板边界的形成和神经嵴发育[49]。对于胃肠道发育, NKX6-3 有以下几个功能: 1) 通过促进胃分化标志物 SOX2 的表达, 以及抑制肠分化标志物尾型同源盒转录因子 2 (caudal-type homeobox protein 2, CDX2)和黏蛋白 2 (mucin 2, Muc2)的表达, 参与胃黏膜细胞命运决定[50]; 2) 通过下调胃上皮细胞 NF-κB 和 DNA 甲基转移酶 1 (DNA methyltransferase 1, DNMT1)的表达, 诱导抗氧化基因 *HAC-E1* 表达, 同时抑制活性氧(reactive oxygen species, ROS)的产生, 从而增强胃黏膜屏障功能[51]; 3) 通过抑制 γ-分泌酶复合物的形成和载脂蛋白 E (apolipoprotein E, APOE)、β 分泌酶 1 (beta-secretase 1, BACE1)的表达, 调节 β 淀粉样蛋白(amyloid β-protein, Aβ)的积累和寡聚, 从而防止胃黏膜萎缩, 并通过调节 APOE 诱导的 NF-κB 的活性和细胞因子的表达来抑制胃黏膜炎症[52]。

2.2 NKX 蛋白在肿瘤发生过程中的作用

NKX2-1 参与肺癌发生过程。*NKX2-1* 基因的突变、高甲基化以及表观遗传沉默机制, 如 mi-

表 3　NKX 同源框蛋白的生理功能

Table 3　Physiological functions of NKX homeobox proteins

Family	Protein name	Physiological function
NK1	NKX1-1	Provides positional information for the formation of body patterns in the body wall muscles[16]
	NKX1-2	1) Regulates the formation of mesoderm[17] 2) Regulates differentiation of adipocytes and osteoblasts, and promotes adipogenesis[18]
NK2.1	NKX2-1	1) Regulates morphogenesis and differentiation of epithelial cells of lung[19] 2) Establishes and maintains phenotype, function, internal environmental balance, and tissue differentiation of thyroid[19] 3) Regulates the formation of astrocytes[9, 21] 4) Associated with non-TRU type lung adenocarcinoma[53] 5) Inhibits the growth of 95D cells in lung cancer[54]
	NKX2-4	Affects the differentiation of megakaryocytes and erythrocytes, and is associated with the formation of AML subtypes[10]
NK2.2	NKX2-2	1) Regulates pattern formation of the V3 domain of the embryonic neural tube[23] 2) Regulates the development and maturation of oligodendrocytes[23] 3) Involved in determining the fate of motor neurons in the hindbrain and viscera[24] 4) Involved in determining the fate of islet progenitor cells, and is associated with severe diabetes and very low birth weight infants, childhood obesity, and developmental delay[8] 5) Inhibits the growth and metastasis of osteosarcoma cells[55]
	NKX2-8	1) Involved in early heart and lung development[25] 2) Involved in many cancers, such as ESCC[56], HCC[57], IBC[58], BUC[59] and EOC[60]
NK3	NKX3-1	1) Involved in the fate of prostate epithelial cells and maintains normal prostate secretory function[26] 2) Involved in many cancers, such as PCa, OSCC[61] and NPC[62]
	NKX3-2	1) Regulates the development of cartilage and spine[27] 2) Involved in the pattern formation of visceral mesoderm, and affects the development of gastric stroma and spleen[29]
NK4	NKX2-3	1) Inhibits the formation of restenosis induced by intimal hyperplasia[30] 2) Associated with the development of inflammatory bowel disease[31] 3) Involved in the development and differentiation of peripheral lymphoid organs[32], and is associated with lymphomatosis[63]
	NKX2-5	1) Involved in cardiac morphogenesis, and its mutation can lead to many heart diseases, such as tetralogy of Fallot, double outlet of right ventricle[33], ventricular septal defect[34] and atrial fibrillation[35] 2) Regulates the differentiation, proliferation and survival of pharyngeal endodermal cells[36] 3) Affects differentiation and development of thyroid, and is associated with congenital primary hypothyroidism[37]
	NKX2-6	1) Involved in cardiac morphogenesis, and its mutation can lead to many heart diseases, such as tetralogy of Fallot, double outlet of right ventricle[33], ventricular septal defect[34] and atrial fibrillation[35] 2) Regulates the differentiation, proliferation and survival of pharyngeal endodermal cells[36]
NK5	HMX1	Regulates craniofacial development, affects retinal differentiation, and is associated with congenital microphthalmos[38]
	HMX2	1) Regulates development of inner ear[40] 2) Regulates differentiation of hemopoietic stem cells, and is associated with AML formation[41]
	HMX3	1) Regulates development of inner ear[40] 2) Regulates differentiation of hemopoietic stem cells, and is associated with AML formation[41]
NK6	NKX6-1	1) Regulates the development of midbrain dopaminergic neurons, affects normal brain function, and is involved in the formation of serious neurological diseases such as Parkinson's disease[42] 2) Required to activate the differentiation of pancreatic progenitor cells into β-cells, and is associated with the formation of type 2 diabetes mellitus[43] 3) Regulates numbers of pancreatic α cell and secretion of glucagon[44]
	NKX6-2	1) Regulates the development of spinal neurons[11] 2) Regulates the identification and differentiation of basal ganglia neuronal progenitor cells in telencephalon, midbrain and diencephalon[45] 3) Regulates maturation of oligodendrocytes and development of myelin sheath[46] 4) Involved in determining the fate of α cells and β cells[48] 5) Inhibits proliferation and invasion of gastric cancer cells[64]
	NKX6-3	1) Promotes the formation of neural plate boundaries and development of neural crest[49] 2) Involved in determining the fate of gastric mucosa cells[50] 3) Inhibits ROS production, thereby enhancing gastric mucosal barrier function[51] 4) Inhibits inflammation of gastric mucosa[52] 5) Involved in occurrence of gastric cancer in many ways[65]

RNA 和组蛋白修饰, 会导致非终末呼吸单位(terminal respiratory unit, TRU)型肺腺癌[53]; 其还可以通过上调肿瘤抑制因子 miR-7 的体内含量显著抑制人肺癌 95D 细胞的生长[54], 因此 *NKX2-1* 可以作为肺癌基因治疗的潜在靶点。

NKX2-2 通过介导 V 型胶原 α2 (collagen alpha 2 (V) chain, COL5A2)、尿激酶型纤溶酶原激活物(urokinase-type plasminogen activator, u-PA)、脑信号蛋白 7A (semaphorin 7A, SEMA7A)和鞘氨醇-1-磷酸受体 1 (sphingosine-1-phosphate receptor 1, S1PR1)的转录下调, 降低体外骨肉瘤细胞的迁移、侵袭和增殖集落的形成, 同时可抑制体内肿瘤的生长和转移, 为骨肉瘤提供了一系列潜在的治疗靶点[55]。

NKX2-8 参与多种癌症发生。在食管鳞状细胞癌(esophageal squamous cell carcinoma, ESCC)中, NKX2-8 通过负调控血管内皮生长因子 C (vascular endothelial growth factor C, VEGFC)和 NF-κB的结合配体 A 激酶相互作用蛋白 1 (A kinase-interacting protein 1, AKIP1)抑制肿瘤发生[56]; 在肝细胞癌(hepatocellular carcinoma, HCC)中, NKX2-8 通过抑制癌细胞增殖和集落形成抑制肿瘤发生[57]; 在浸润性膀胱癌(invasive bladder carcinoma, IBC)中, NKX2-8 通过正调控周期蛋白激酶抑制因子 p27Kip1 和负调控周期蛋白 D1、叉头框蛋白 O3 (forkhead box protein O3, FOXO3)和丝裂原活化蛋白激酶激酶/胞外信号调节激酶(mitogen-activated protein kinase kinase/extracellular signal-regulated kinase, MEK/ERK)通路抑制肿瘤发生[58]; 在膀胱尿路上皮细胞癌(bladder urothelial carcinoma, BUC)中, NKX2-8 通过负调控上皮-间质转化(epithelial-mesenchymal transition, EMT)诱导剂 Twist1 的表达抑制肿瘤发生[59]; 在上皮性卵巢癌(epithelial ovarian cancer, EOC)中, NKX2-8 通过负调控长链脂肪醇氧化酶(long-chain fatty alcohol oxidase, FAO)通路的肉毒碱棕榈酰转移酶(carnitine palmitoyltransferase, CPT) 1A 和 2 抑制肿瘤发生[60]。综上可知, NKX2-8 可以作为多种癌症临床预后的生物标志物和潜在的治疗靶点。

NKX3-1 是前列腺癌(prostate cancer, PCa)的公认标志物, 除此以外, NKX3-1 的缺失导致口腔鳞状细胞癌(oral squamous cell carcinoma, OSCC)隐匿性颈部淋巴结转移(lymph node metastasis, LNM)[61], 并且 NKX3-1 可抑制鼻咽癌 (nasopharyngeal carcinoma, NPC)癌细胞活力[62]。

NKX2-3 通过 B 细胞受体信号转导激活多种整合素(LFA-1 和 VLA-4)、黏附分子(ICAM-1 和 MadCAM-1)和趋化因子受体 CXCR4, 增强 B 细胞向脾和结外淋巴组织的迁移、极化和归巢, 最终触发 NF-κB 和 PI3K/Akt 通路, 驱动恶性转化, 导致淋巴瘤发生, 这为人类边缘区 B 细胞淋巴瘤的研究和治疗提供了有效依据[63]。

NKX6-2 通过促进胃动蛋白(gastrokine 2, GKN2)的表达抑制胃癌细胞增殖和侵袭, 因此可作为临床胃癌患者潜在的诊断和治疗靶点[64]。此外, NKX6-3 也可以多种途径参与胃癌的发生, 比如通过下调 Wnt/β-catenin 和 Rho-GTPase 通路抑制癌细胞的迁移和侵袭[65]; 通过调控 DNA 复制因子 CDT1 和 RNA 聚合酶Ⅰ亚基 A1 (RNA polymeraseⅠsubunit A1, RPA1)的表达, 调节细胞周期进展、癌基因和抑癌基因组的 DNA 复制与 DNA 损伤修复, 从而影响肿瘤相关蛋白质的表达[66]等。

3 总结与展望

NKX 蛋白作为调控胚胎发育和肿瘤发生过程中重要的转录因子历年来得到了广泛研究。基于前述资料可知, NKX 蛋白对于神经系统、心脏、甲状腺、肺、胃肠道、胰腺等的发育起关键的调控作用并参与多种相关疾病的生成, 同时与甲状腺癌、肺癌、前列腺癌等肿瘤的发生相关。现有研究揭示了一部分 NKX 蛋白的调控网络及其信号通路, 比如: NKX1-2 通过参与 Wnt/β-catenin 信号通路下游调控网络调节神经中、内胚层的形成[17]; NKX2-4 和 NKX2-3 通过调节巨核细胞和红细胞分化相关基因调控网络参与 AML 的发生[10]; HMX2 和 HMX3 通过参与 IL-7 和 Wnt 信号通路下游基因调控网络调节骨髓分化[41]。然而, 大部分 *NKX* 基因的基因型虽然与表型的关系明确, 但是缺乏包括转录组和表达谱分析等在内的多组学分析, 以及模式动物疾病模型构建等的研究, 这使得 *NKX* 基因突变或缺失导致相关疾病的致病机理探究与治疗严重受限。对此, 后续工作应基于各 NKX 蛋白家族的不同临床表现, 加速推进相应的多组学分析和模式生物疾病模型的构建等, 以早日阐明 NKX 蛋白参与调控的具体分子机制, 为相关疾病的临床治疗提供理论依据。值得关注的是, NKX 蛋白的体内功能存在广泛交叉现象, 比如: NKX2-1 与 NKX2-4 均参与下丘脑发育[22];

NKX2-5 和 NKX2-6 共同调控咽部发育[36]; HMX2 和 HMX3 在内耳发育中存在功能冗余[40]; NKX6-2 与 NKX6-1 在神经元发育中具有冗余作用, 甚至 NKX6-1 会抑制 NKX6-2 的表达[67]。因此, 了解 NKX 蛋白功能交叉或功能冗余背后的调控关系, 有利于更好地解释每个基因存在的重要意义。

另外, 本文综述了 NKX 蛋白在生化和结构方面的研究, 简要整理了 NKX 蛋白的结构特征及其与 DNA 的精细结合模式。目前, NKX2-1、NKX3-1 和 NKX2-5 的三维结构模型已被报道, 其中只有 NKX2-5-DNA 复合物中的 DNA 为内源性基因片段, 并且研究显示 NKX2-5 与心脏病形成相关的错义突变会影响其与核酸的结合[15]。遗憾的是, NKX3-1 等其他结构中的 DNA 均非内源性基因片段, 因而不足以解释其在体内的互作模式, 而且, NKX2-4 等若干 NKX 蛋白更是缺乏其识别核酸元件的结构与生化研究, 这严重阻碍了人们对这些蛋白质致病机制的认识。此外, NKX 蛋白相应的临床病理突变体对自身核酸底物识别的特异性及强度是否改变, 病理突变是否以及如何影响转录活性, 多个 NKX 蛋白之间的协同效应如何实现, 等等, 这一系列问题均有待进一步深入探究。

参考文献(References):

[1] MCGINNIS W, LEVINE M S, HAFEN E, *et al*. A conserved DNA sequence in homoeotic genes of the *Drosophila* antennapedia and bithorax complexes[J]. Nature, 1984, 308(5958): 428-433.

[2] SEIMIYA M, ISHIGURO H, MIURA K, *et al*. Homeobox-containing genes in the most primitive metazoa, the sponges[J]. European Journal of Biochemistry, 1994, 221(1): 219-225.

[3] CARRASCO A E, MCGINNIS W, GEHRING W J, *et al*. Cloning of an *X. laevis* gene expressed during early embryogenesis coding for a peptide region homologous to *Drosophila* homeotic genes[J]. Cell, 1984, 37(2): 409-414.

[4] LEVINE M, RUBIN G M, TJIAN R. Human DNA sequences homologous to a protein coding region conserved between homeotic genes of *Drosophila*[J]. Cell, 1984, 38(3): 667-673.

[5] GEHRING W J, QIAN Y Q, BILLETER M, *et al*. Homeodomain-DNA recognition[J]. Cell, 1994, 78(2): 211-223.

[6] BÜRGLIN T R, AFFOLTER M. Homeodomain proteins: an update[J]. Chromosoma, 2016, 125(3): 497-521.

[7] KIM Y, NIRENBERG M. *Drosophila* NK-homeobox genes[J]. Proceedings of the National Academy of Sciences of USA, 1989, 86(20): 7716-7720.

[8] AUERBACH A, COHEN A, OFEK SHLOMAI N, *et al*. *NKX2-2* mutation causes congenital diabetes and infantile obesity with paradoxical glucose-induced ghrelin secretion[J]. The Journal of Clinical Endocrinology and Metabolism, 2020, 105(11): dgaa563.

[9] GUAN L, ZHAO X, TANG L, *et al*. Thyroid transcription factor-1: structure, expression, function and its relationship with disease[J]. BioMed Research International, 2021, 2021: 9957209.

[10] NAGEL S, POMMERENKE C, MEYER C, *et al*. NKL homeobox genes *NKX2-3* and *NKX2-4* deregulate megakaryocytic-erythroid cell differentiation in AML[J]. International Journal of Molecular Sciences, 2021, 22(21): 11434.

[11] TOCH M, HARRIS A, SCHAKMAN O, *et al*. Onecut-dependent Nkx6.2 transcription factor expression is required for proper formation and activity of spinal locomotor circuits[J]. Scientific Reports, 2020, 10: 996.

[12] HOLLAND P W H, BOOTH H A F, BRUFORD E A. Classification and nomenclature of all human homeobox genes[J]. BMC Biology, 2007, 5: 47.

[13] GRUSCHUS J M, TSAO D H, WANG L H, *et al*. Interactions of the vnd/NK-2 homeodomain with DNA by nuclear magnetic resonance spectroscopy: basis of binding specificity[J]. Biochemistry, 1997, 36(18): 5372-5380.

[14] ZHENG S L, JU J H, CHANG B L, *et al*. Germ-line mutation of *NKX3.1* cosegregates with hereditary prostate cancer and alters the homeodomain structure and function[J]. Cancer Research, 2006, 66(1): 69-77.

[15] PRADHAN L, GENIS C, SCONE P, *et al*. Crystal structure of the human NKX2.5 homeodomain in complex with DNA target[J]. Biochemistry, 2012, 51(32): 6312-6319.

[16] SCIMONE M L, COTE L E, REDDIEN P W. Orthogonal muscle fibres have different instructive roles in planarian regeneration[J]. Nature, 2017, 551(7682): 623-628.

[17] TAMASHIRO D A A, ALARCON V B, MARIKAWA Y. Nkx1-2 is a transcriptional repressor and is essential for the activation of Brachyury in P19 mouse embryonal carcinoma cell[J]. Differentiation, 2012, 83(5): 282-292.

[18] CHEN N, SCHILL R L, O'DONNELL M, *et al*. The transcription factor NKX1-2 promotes adipogenesis and may contribute to a balance between adipocyte and osteoblast differentiation[J]. Journal of Biological Chemistry, 2019, 294(48): 18408-18420.

[19] PHELPS C A, LAI S C, MU D. Roles of thyroid transcription factor 1 in lung cancer biology[J]. Vitamins and Hormones, 2018, 106: 517-544.

[20] MONTANELLI L, TONACCHERA M. Genetics and phenomics of hypothyroidism and thyroid dys- and agenesis due to PAX8 and TTF1 mutations[J]. Molecular and Cellular Endocrinology, 2010, 322(1/2): 64-71.

[21] MINOCHA S, VALLOTON D, ARSENIJEVIC Y, *et al*. Nkx2.1 regulates the generation of telencephalic astrocytes during embryonic development[J]. Scientific Reports, 2017, 7: 43093.

[22] MANOLI M, DRIEVER W. *Nkx*2.1 and *nkx*2.4 genes function partially redundant during development of the zebrafish hypothalamus, preoptic region, and pallidum[J]. Frontiers in Neuroanatomy, 2014, 8: 145.

[23] MARIYATH M P M, SHAHI M H, FARHEEN S, *et al*. Novel homeodomain transcription factor Nkx2.2 in the brain tumor development[J]. Current Cancer Drug Targets, 2020, 20(5): 335-340.

[24] JARRAR W, DIAS J M, ERICSON J, *et al*. Nkx2.2 and Nkx2.9 are the key regulators to determine cell fate of branchial and visceral motor neurons in caudal hindbrain[J]. PLoS One, 2015, 10(4): e0124408.

[25] TIAN J M, MAHMOOD R, HNASKO R, *et al*. Loss of Nkx2.8 deregulates progenitor cells in the large airways and leads to dysplasia[J]. Cancer Research, 2006, 66(21): 10399-10407.

[26] LE MAGNEN C, VIRK R K, DUTTA A, *et al*. Cooperation of loss of NKX3.1 and inflammation in prostate cancer initiation[J]. Disease Models & Mechanisms, 2018, 11(11): dmm035139.

[27] WALDMANN L, LEYHR J, ZHANG H Q, *et al*. The broad role of Nkx3.2 in the development of the zebrafish axial skeleton[J]. PLoS One, 2021, 16(8): e0255953.

[28] SIMSEK-KIPER P O, KOSUKCU C, AKGUN-DOGAN O, *et al*. A novel NKX3-2 mutation associated with perinatal lethal phenotype of spondylo-megaepiphyseal-metaphyseal dysplasia in a neonate[J]. European Journal of Medical Genetics, 2019, 62(1): 21-26.

[29] LETTICE L, HECKSHER-SØRENSEN J, HILL R. The role of *Bapx*1 (*Nkx*3.2) in the development and evolution of the axial skeleton[J]. Journal of Anatomy, 2001, 199(1/2): 181-187.

[30] ZHENG H J, ZHAI W C, ZHONG C B, *et al*. Nkx2-3 induces autophagy inhibiting proliferation and migration of vascular smooth muscle cells via AMPK/mTOR signaling pathway[J]. Journal of Cellular Physiology, 2021, 236(11): 7342-7355.

[31] YU W, HEGARTY J P, BERG A, *et al*. NKX2-3 transcriptional regulation of endothelin-1 and VEGF signaling in human intestinal microvascular endothelial cells[J]. PLoS One, 2011, 6(5): e20454.

[32] VOJKOVICS D, KELLERMAYER Z, KAJTÁR B, *et al*. Nkx2-3: a slippery slope from development through inflammation toward hematopoietic malignancies[J]. Biomarker Insights, 2018, 13: 1177271918757480.

[33] ZHAO L, NI S H, LIU X Y, *et al*. Prevalence and spectrum of Nkx2.6 mutations in patients with congenital heart disease[J]. European Journal of Medical Genetics, 2014, 57(10): 579-586.

[34] WANG J, MAO J H, DING K K, *et al*. A novel NKX2.6 mutation associated with congenital ventricular septal defect[J]. Pediatric Cardiology, 2015, 36(3): 646-656.

[35] WANG J, ZHANG D F, SUN Y M, *et al*. NKX2-6 mutation predisposes to familial atrial fibrillation[J]. International Journal of Molecular Medicine, 2014, 34(6): 1581-1590.

[36] TANAKA M, SCHINKE M, LIAO H S, *et al*. Nkx2.5 and Nkx2.6, homologs of *Drosophila* tinman, are required for development of the pharynx[J]. Molecular and Cellular Biology, 2001, 21(13): 4391-4398.

[37] KOSTOPOULOU E, MILIORDOS K, SPILIOTIS B. Genetics of primary congenital hypothyroidism-a review[J]. Hormones, 2021, 20(2): 225-236.

[38] EL FERSIOUI Y, PINTON G, ALLAMAN-PILLET N, *et al*. Hmx1 regulates *urfh*1 expression in the craniofacial region in zebrafish[J]. PLoS One, 2021, 16(1): e0245239.

[39] GILLESPIE R L, URQUHART J, LOVELL S C, *et al*. Abrogation of HMX1 function causes rare oculoauricular syndrome associated with congenital cataract, anterior segment dysgenesis, and retinal dystrophy[J]. Investigative Ophthalmology & Visual Science, 2015, 56(2): 883-891.

[40] ENGLAND S J, CERDA G A, KOWALCHUK A, *et al*. Hmx3a has essential functions in zebrafish spinal cord, ear and lateral line development[J]. Genetics, 2020, 216(4): 1153-1185.

[41] NAGEL S, POMMERENKE C, MEYER C, *et al*. Aberrant expression of NKL homeobox genes *HMX*2 and *HMX*3 interferes with cell differentiation in acute myeloid leukemia[J]. PLoS One, 2020, 15(10): e0240120.

[42] NOURI N, PATEL M J, JOKSIMOVIC M, *et al*. Excessive Wnt/beta-catenin signaling promotes midbrain floor plate neurogenesis, but results in vacillating dopamine progenitors[J]. Molecular and Cellular Neuroscience, 2015, 68: 131-142.

[43] MOIN A S M, BUTLER A E. Alterations in beta cell identity in type 1 and type 2 diabetes[J]. Current Diabetes Reports, 2019, 19(9): 83.

[44] XU S, XU J P. Present status and expectation of aristaless-related homeobox (ARX) in endocrine pancreas[J]. International Journal of Developmental Biology, 2019, 63(11/12): 579-587.

[45] MORENO-BRAVO J A, PEREZ-BALAGUER A, MARTINEZ S, *et al*. Dynamic expression patterns of Nkx6.1 and Nkx6.2 in the developing mes-diencephalic basal plate[J]. Developmental Dynamics, 2010, 239(7): 2094-2101.

[46] CHELBAN V, PATEL N, VANDROVCOVA J, *et al*. Mutations in NKX6-2 cause progressive spastic ataxia and hypomyelination[J]. American Journal of Human Genetics, 2017, 100(6): 969-977.

[47] BALDI C, BERTOLI-AVELLA A M, AL-SANNAA N, *et al*. Expanding the clinical and genetic spectra of NKX6-2-related disorder[J]. Clinical Genetics, 2018, 93(5): 1087-1092.

[48] BINOT A C, MANFROID I, FLASSE L, *et al*. Nkx6.1 and nkx6.2 regulate alpha- and beta-cell formation in zebrafish by acting on pancreatic endocrine progenitor cells[J]. Developmental Biology, 2010, 340(2): 397-407.

[49] ZHANG Z M, SHI Y, ZHAO S H, *et al*. *Xenopus Nkx*6.3 is a neural plate border specifier required for neural crest development[J]. PLoS One, 2014, 9(12): e115165.

[50] YOON J H, CHOI W S, KIM O, *et al*. NKX6.3 controls gastric differentiation and tumorigenesis[J]. Oncotarget, 2015, 6(29): 28425-28439.

[51] YOON J H, KIM O, NAM S W, *et al*. NKX6.3 regulates reactive oxygen species production by suppressing NF-κB and DNMT1 activities in gastric epithelial cells[J]. Scientific Reports, 2017, 7: 2807.

[52] YOON J H, LEE Y S, KIM O, *et al*. NKX6.3 protects against gastric mucosal atrophy by downregulating β-amyloid production[J]. World Journal of Gastroenterology, 2019, 25(3): 330-345.

[53] MATSUBARA D, YOSHIMOTO T, SODA M, *et al*. Reciprocal expression of trefoil factor-1 and thyroid transcription factor-1 in lung adenocarcinomas[J]. Cancer Science, 2020, 111(6): 2183-2195.

[54] LEI L Y, CHEN C, ZHAO J J, *et al*. Targeted expression of miR-7 operated by TTF-1 promoter inhibited the growth of human lung cancer through the NDUFA4 pathway[J]. Molecular Therapy. Nucleic Acids, 2017, 6: 183-197.

[55] CHEN H M, LIU W Q, ZHONG L, *et al*. NKX2-2 suppresses osteosarcoma metastasis and proliferation by downregulating multiple target genes[J]. Journal of Cancer, 2018, 9(17): 3067-3077.

[56] LIN C Y, SONG L B, GONG H, *et al*. Nkx2-8 downregulation promotes angiogenesis and activates NF-κB in esophageal cancer[J]. Cancer Research, 2013, 73(12): 3638-3648.

[57] QU L, DENG B, ZENG Y, *et al*. Decreased expression of the *Nkx*2.8 gene correlates with tumor progression and a poor prognosis in HCC cancer[J]. Cancer Cell International, 2014, 14: 28.

[58] YU C P, ZHANG Z L, LIAO W T, *et al*. The tumor-suppressor gene *Nkx*2.8 suppresses bladder cancer proliferation through upregulation of FOXO3a and inhibition of the MEK/ERK signaling pathway[J]. Carcinogenesis, 2012, 33(3): 678-686.

[59] YU C P, LIU Z W, CHEN Q H, *et al*. Nkx2.8 inhibits epithelial-mesenchymal transition in bladder urothelial carcinoma via transcriptional repression of Twist1[J]. Cancer Research, 2018, 78(5): 1241-1252.

[60] ZHU J R, WU G Y, SONG L B, *et al*. *NKX*2-8 deletion-induced reprogramming of fatty acid metabolism confers chemoresistance in epithelial ovarian cancer[J]. EBioMedicine, 2019, 43: 238-252.

[61] MIYAGUCHI K, UZAWA N, MOGUSHI K, *et al*. Loss of NKX3-1 as a potential marker for an increased risk of occult lymph node metastasis and poor prognosis in oral squamous cell carcinoma[J]. International Journal of Oncology, 2012, 40(6): 1907-1914.

[62] QIAN D, ZHENG W C, CHEN C X, *et al*. Roles of CCNB2 and NKX3-1 in nasopharyngeal carcinoma[J]. Cancer Biotherapy & Radiopharmaceuticals, 2020, 35(3): 208-213.

[63] ROBLES E F, MENA-VARAS M, BARRIO L, *et al*. Homeobox NKX2-3 promotes marginal-zone lymphomagenesis by activating B-cell receptor signalling and shaping lymphocyte dynamics[J]. Nature Communications, 2016, 7: 11889.

[64] DAI J, PENG T L, YU X D. NK6 homeobox 2 regulated gastrokin-2 suppresses gastric cancer cell proliferation and invasion via Akt signaling pathway[J]. Cell Biochemistry and Biophysics, 2021, 79(1): 123-131.

[65] YOON J H, EUN J W, CHOI W S, *et al*. NKX6.3 is a transcription factor for Wnt/β-catenin and Rho-GTPase signaling-related genes to suppress gastric cancer progression[J]. EBioMedicine, 2016, 9: 97-109.

[66] YOON J H, EUN J W, ASHKTORAB H, *et al*. Depletion of NK6 homeobox 3 (NKX6.3) causes gastric carcinogenesis through copy number alterations by inducing impairment of DNA replication and repair regulation[J]. Oncogenesis, 2021, 10(12): 85.

[67] VALLSTEDT A, MUHR J, PATTYN A, *et al*. Different levels of repressor activity assign redundant and specific roles to *Nkx*6 genes in motor neuron and interneuron specification[J]. Neuron, 2001, 31(5): 743-755.

(上接第 30 页)

[12] DAMIANI I, PAULY N, PUPPO A, *et al*. Reactive oxygen species and nitric oxide control early steps of the legume-rhizobium symbiotic interaction[J]. Frontiers in Plant Science, 2016, 7: 454.

[13] 闻静, 刘俊, 段华平, 等. 三叶青 2 个 ThBLH 转录因子 cDNA 的克隆及其对光质与光周期信号的响应[J]. 河南农业大学学报(WEN Jing, LIU Jun, DUAN Huaping, *et al*. Cloning of two ThBLH transcription factors cDNA of *Tetrastigma hemsleyanum* Diels et Gilg and its response to light quality and photoperiod signals[J]. Journal of Henan Agricultural University), 2021, 55(6): 1065-1073.

[14] KOCH M, VELARDE M, HARRISON M D, *et al*. Crystal structures of oxidized and reduced stellacyanin from horseradish roots[J]. Journal of the American Chemical Society, 2005, 127(1): 158-166.

[15] SUN Y L, WU Z F, WANG Y J, *et al*. Identification of phytocyanin gene family in legume plants and their involvement in nodulation of *Medicago truncatula*[J]. Plant & Cell Physiology, 2019, 60(4): 900-915.

[16] MASHIGUCHI K, ASAMI T, SUZUKI Y. Genome-wide identification, structure and expression studies, and mutant collection of 22 early nodulin-like protein genes in *Arabidopsis*[J]. Bioscience, Biotechnology, and Biochemistry, 2009, 73(11): 2452-2459.

[17] MASHIGUCHI K, YAMAGUCHI I, SUZUKI Y. Isolation and identification of glycosylphosphatidylinositol-anchored arabinogalactan proteins and novel beta-glucosyl Yariv-reactive proteins from seeds of rice (*Oryza sativa*)[J]. Plant & Cell Physiology, 2004, 45(12): 1817-1829.

[18] HIRSCH A M, BHUVANESWARI T V, TORREY J G, *et al*. Early nodulin genes are induced in alfalfa root outgrowths elicited by auxin transport inhibitors[J]. Proceedings of the National Academy of Sciences of USA, 1989, 86(4): 1244-1248.

[19] SCHERES B, VAN EENGELEN F, VEN DER KNAAP E, *et al*. Sequential induction of nodulin gene expression in the developing pea nodule[J]. Plant Cell, 1990, 2(8): 687-700.

[20] CHEN L Q, QU X Q, HOU B H, *et al*. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport[J]. Science, 2012, 335(6065): 207-211.

[21] LI B, LI Y Y, WU H M, *et al*. Root exudates drive interspecific facilitation by enhancing nodulation and N_2 fixation[J]. Proceedings of the National Academy of Sciences of USA, 2016, 113(23): 6496-6501.

[22] REDDY P M, AGGARWAL R K, RAMOS M C, *et al*. Widespread occurrence of the homologues of the early nodulin (*ENOD*) genes in *Oryza* species and related grasses[J]. Biochemical and Biophysical Research Communications, 1999, 258(1): 148-154.

[23] HOU Y N, GUO X Y, CYPRYS P, *et al*. Maternal ENODLs are required for pollen tube reception in *Arabidopsis*[J]. Current Biology, 2016, 26(17): 2343-2350.