NuMA蛋白质的生物学作用

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摘要 NuMA(nuclear mitotic apparatus)是一个高分子量的细胞核有丝分裂器蛋白。自1980年发现至今已有30多年的历史。研究发现, NuMA对细胞有丝分裂过程中纺锤体的形成和结构维持、细胞分裂后期核重组均发挥重要作用。NuMA的过表达与恶性肿瘤发生发展相关, NuMA的降解将导致细胞分裂异常及细胞核骨架的分解。该文将对NuMA的表达及定位、可变剪接体、相互作用蛋白质及其在有丝分裂、不对称分裂及核移植胚胎早期发育等过程中的功能进行了系统综述。

关键词 细胞核有丝分裂器蛋白; 有丝分裂; 恶性肿瘤; 可变剪接体; 核移植

The Biological Functions of NuMA Protein

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Abstract Nuclear mitotic apparatus (NuMA) is a nuclear mitotic apparatus protein with high molecular weight. It has been more than 30 years since it was discovered at the first time in 1980. It has been found that NuMA plays important roles in mitotic spindle organization and maintenance, as well as post-mitotic nuclear reorganization. Moreover, overexpression of NuMA was closely related to the occurrence and development of multiple malignant tumors and the degradation of NuMA could lead to abnormal cell division and the decomposition of nuclear scaffold. In current paper, the expression and localization, alternatively splicing isoforms and interaction proteins of NuMA, as well as the progress in functions of NuMA in mitosis, asymmetric division and early development of nuclear transfer embryos were systematically reviewed.

Keywords nuclear mitotic apparatus; mitosis; malignant tumor; isoforms; nuclear transfer

NuMA(nuclear mitotic apparatus)是一个高分子量的细胞核有丝分裂器蛋白, 最早于1980年由Lydersen和Pettijohn发现并命名[1]。NuMA在分裂间期有专一的细胞核定位, 但在分裂期与纺锤极的功能相关。在NuMA序列被鉴定之前, NuMA多肽已经被5个不同的实验室发现, 并分别被命名为centrophilin、SPN(spindle pole-nucleus)和SP-H(human spindle pole antigen)[2-4]。后续研究发现, NuMA是中心体蛋白的乘客蛋白中一类特殊蛋白质, 在分裂间期定位于细胞核, 行使与微管不相关的功能。在分裂期, NuMA蛋白在胞质驱动蛋白和动力蛋白提供驱动力的作用下, 转位到中心体附近区域参与组装纺锤体和维持纺锤体稳定的功能[5]。NuMA主要包含一个球形头部(N-端)、一个球形尾部(C-端)及中间包含1 500个氨基酸的非连续卷曲结构组成(图1A)[6]。NuMA包含长、中、短三类不同的可变剪接体(splicing

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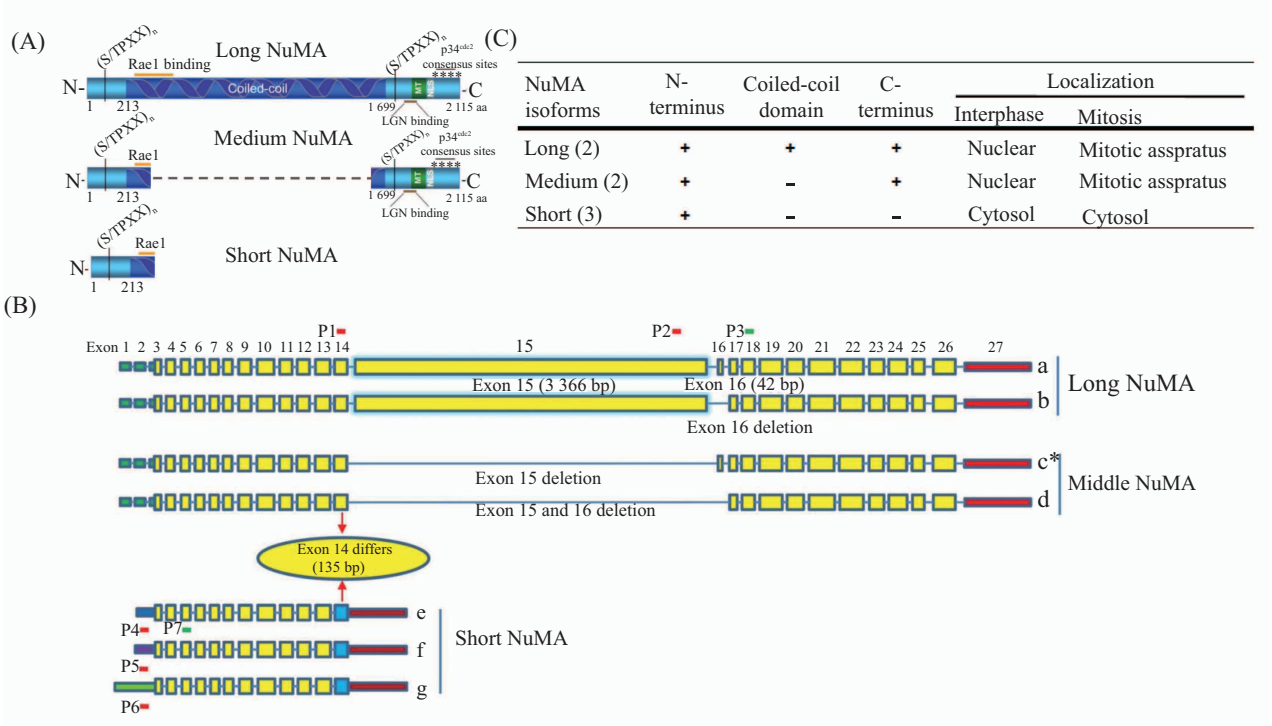
isoforms), 其中, 长、中型NuMA的N-和C-末端结构域一致, 且包含与有丝分裂及核定位相关的保守模体(motif), 可能行使与RNA剪切及有丝分裂相关的功能。而短型NuMA因缺少coil-coiled及C-端结构域, 整个细胞周期都定位于细胞质, 并且可能行使肿瘤抑制方面功能(图1)[7]。此外, 近些年的研究表明, NuMA还在不对称分裂[8]、胚胎移植及肿瘤等多种人类疾病中[9-10]发挥了重要作用。本文将依据现有文献研究结果, 系统阐述NuMA1的基因结构、蛋白质表达及其与人类疾病(包括肿瘤)的密切关系。

1 NuMA的基因结构

人类NuMA由单基因*NuMA1*表达, *NuMA1*是一个在哺乳动物细胞最常见且表达丰度较高的单基因, 每个细胞大约有2×10^{5}个拷贝, 该基因定位于11q13, 全长约为77.7 Kb, 其mRNA全长7 183 bp, 能编码分子量



NuMA isoforms	N-terminus	Coiled-coil domain	C-terminus	Localization	
				Interphase	Mitosis
Long (2)	+	+	+	Nuclear	Mitotic asspratus
Medium (2)	+	–	+	Nuclear	Mitotic asspratus
Short (3)	+	–	–	Cytosol	Cytosol

A: 依据现有文献报道, NuMA剪接体分为长、中和短三类, 其中, 长型NuMA包括氨基球形末端、羧基球形末端及中间coiled-coil结构域, 中型NuMA缺少中间coiled-coil结构域, 短型NuMA仅包括氨基球形末端结构域; B: 不同NuMA剪接体由不同NuMA1转录本转录表达。长型NuMA由转录本a和b转录表达, 两转录本之间仅有42 bp(第16号外显子)序列差异, 中型NuMA由转录本c和d转录表达, 两转录本之间也仅有42 bp序列差异, 且*表示本课题组新鉴定出的转录本(UCSC Genome Browser和NCBI数据库均未记载), 短型NuMA由转录本e、f和g转录表达, 三个转录本仅仅在5′UTR区域有序列差异, 开放读码框完全一致, 编码同一种蛋白, 且与长型和中型NuMA相比, exon14有135 bp的序列差异, P1-P3引物对用于检测转录本a和b, P2-P3引物对用于检测转录本c和d, P4(P5/P6)-P7引物对分别用于检测转录本e、f和g, 黄色填充的矩形表示编码蛋白的外显子, 其他显色填充的矩形表示5′或3′非翻译区的外显子; C: 不同NuMA剪接体的亚细胞定位不同。长型和中型NuMA在有丝分裂间期和分裂期分别定位于细胞核和有丝分裂器, 而短型NuMA在整个细胞周期都定位于细胞质, 提示短型NuMA有不同的生物学功能。

A: based on existing literatures, NuMA isoforms could be divided into three categories: long, medium and short NuMA, of which long NuMA comprised of N-terminus, C-terminus and coiled-coil domain; medium NuMA lack of coiled-coil domain and short NuMA only contains N-terminus domain. B: different NuMA isoforms are transcribed from different NuMA1 transcripts. Long NuMA is transcribed and translated from the transcript a and b (only 42 bp sequence bias between two transcripts); Medium NuMA is transcribed and translated from the transcript c and d (only 42 bp sequence bias between two transcripts); Short NuMA is transcribed and translated from the transcript e, f and g, among which these transcripts have the same open reading frame encoding identical protein while they differ in 5′UTR region sequences. Moreover, compared with long and medium NuMA, 135 bp sequence bias in exon14 is reported. P1-P3 primer pair is designed to detect transcripts of a and b; P2-P3 primer pair is designed to detect transcripts of c and d; While P4 (P5/P6)-P7 primer pairs are designed to detect transcripts of e, f and g. * represents a novel identified transcript which is not recorded in UCSC Genome Browser and the NCBI database. Rectangulars filled with yellow represent exons for protein encoding and rectangulars filled with other colors represent 5′ or 3′ untranslated regions; C: NuMA isoforms have different localization patterns. Long and medium NuMA were distributed in nuclear during interphase and translocated to mitotic apparatus in mitosis, while short NuMA localized in the cytoplasm during the whole cell cycle, suggesting that short NuMA could have different biological functions from long and medium NuMA.

图1 NuMA不同剪接体的基因结构模式图及亚细胞分布(根据参考文献[6]修改)

Fig.1 Gene structure schema and subcellular distribution of NuMA isoforms (modified from reference [6])

约为240 kDa的蛋白质。该基因在生物进化进程中出现得比较晚, 目前只在非洲爪蟾中鉴定出NuMA同源蛋白质X-NuMA, 并且与人类NuMA序列相似度只有37%[11]。NuMA主要包含一个球形头部(N-端), 一个球形尾部(C-端)及中间包含1 500个氨基酸的非连续卷曲结构组成(图1A)。在C-端包含许多与有丝分裂相关的保守模体, 比如在1 971~1 991 aa位置含有一个核定位信号(nuclear localization signal, NLS)[12], 它对于NuMA在有丝分裂间期定位于细胞核非常关键。NuMA有两个缺少核定位信号的可变剪接体, 它们在有丝分裂间期无法定位到细胞核[13]。在1 900~1 971 aa位置含有微管结合模体, NuMA通过该模体与微管相结合, 并在微管蛋白提供驱动力作用下于有丝分裂期转位到中心体附近, 参与组装纺锤体和维持纺锤体稳定的功能[14]。在1 878~1 910 aa位置包含LGN[也称为GPMS2(G-protein signaling modulator 2)]的结合模体, LGN是LGN/Pins蛋白质家族的成员之一, 它在分裂期能够与NuMA C-端结构域结合, 而这个结合位点正好与微管结合位点重叠, 与微管竞争性结合NuMA, 负调控纺锤体的组装[15]。LGN与微管相互竞争的特性对于细胞内聚合微管和游离微管的动态平衡至关重要。在2 000、2 040和2 091 aa位置有三个苏氨酸磷酸化位点, 在2 072 aa位置有一个丝氨酸磷酸化位点[16]。这四个位置的氨基酸都是被$p34^{cdc2}$磷酸化的, 它们的磷酸化修饰对于NuMA进入和离开有丝分裂期非常重要。其中, 任意一个位点发生突变都会导致NuMA不能定位到纺锤极, 无法起始纺锤体的组装, 而是定位到质膜, 参与不对称分裂过程。此外, 在两个球形末端结构域包含一些调控蛋白特有的保守模体S/TPXX, 这些序列主要用于结合DNA[17]。并且, 在coiled-coil区域的前半部(325~829 aa)含有一个信使RNA输出因子Rae1的结合结构域, Rae1主要介导NuMA氨基端与微管相结合, 对于维持纺锤体的稳定很重要。研究表明, RNAi或者过表达Rae1都将导致纺锤体出现多极现象, 并且可以借助NuMA过表达或RNAi逆转Rae1的负面效应[18]。

2 NuMA的表达和定位

前期研究表明, NuMA蛋白质在爪蟾[11]、猪[19]、人[20]等有丝分裂间期, 定位于细胞核内。晚G_2期伴随着细胞核膜崩解, NuMA高度磷酸化转位到细胞质内, 并在中心体附近聚集, 于细胞有丝分裂中期凝集呈新月状分布于纺锤体两极。细胞有丝分裂后期, NuMA分布于分离的染色体外侧。NuMA在细胞有丝分裂末期随染色体进入新生的子细胞内, 并重新定位于细胞核内。然而, NuMA并非在所有类型细胞中都有表达, 如小鼠精子细胞、粒细胞、分化的胃上皮细胞和骨骼肌细胞等未检测到NuMA表达[21], 兔成纤维细胞有丝分裂间期也未见NuMA的表达[22]。在人类也发现了数种细胞存在NuMA缺如的现象, 例如人类乳癌细胞MCF-7、表皮角蛋白细胞、精原细胞和一些神经元等[21,23]。NuMA蛋白质缺失很可能由于特殊的翻译后修饰和蛋白降解等原因所致。

此外, Tang等[24]在1993年应用特异性的抗NuMA抗体进行免疫印迹实验识别出三个条带, 根据分子量大小将这三个条带分别命名为NuMA-l(230 kDa)、NuMA-m(195 kDa)和NuMA-s(194 kDa)。随后, 他们又描述了这三种剪接体(isoforms)的亚细胞定位: NuMA-l在间期定位于细胞核, 分裂期定位于纺锤体两极; NuMA-m和NuMA-s在有丝分裂间期定位于细胞质, 主要位于中心体区域, 分裂期也定位在纺锤体两极[25]。本实验室前期研究发现, NuMA蛋白包括长、中和短三类剪接体, 其中, 长型和中型NuMA在间期定位于细胞核, 分裂期定位于纺锤体两极; 而短型NuMA在整个细胞周期均定位于细胞质(图1C)[7]。

3 NuMA相互作用的蛋白质

前期研究采用酵母双杂交(yeast two hybrid, Y2H)、免疫沉淀(immunoprecipitation, IP)或蛋白芯片等技术手段寻找到一些与NuMA相互作用的蛋白。截止目前, 已经证实与NuMA相互作用且与细胞分裂相关的蛋白有二十多种(表1)[6]。此外, NuMA还能与一些疾病相关的蛋白质相互作用。非红系细胞内Protein 4.1的剪接体Protein 4.1N与NuMA相互作用进而增强神经生长因子的抗增殖活性[26-28]。端锚聚合酶与NuMA直接相互作用, 并且在有丝分裂纺锤极处共定位[29]。在急性早幼粒细胞白血病细胞里, NuMA与视黄酸受体α形成融合基因, 并表达融合蛋白NuMA-RARα, 此融合蛋白能够与类X受体α相互作用并作用于转基因小鼠白血病细胞的发育过程[30]。此外, 聚腺苷二磷酸核糖能够与NuMA相互作用并促进有丝分裂纺锤极的组装[31]。高风险性的人乳头瘤病毒型蛋白16E7与NuMA相互作用将会导致有丝分裂前中期染色体排列异常[32]。动力蛋白5

表1 NuMA相互作用蛋白质汇总

Table 1 Summary of NuMA interacting proteins

细胞时相 Stage	相互作用蛋白质 Interacting partner	与NuMA互作区域 NuMA interacting region	参考文献 References
Interphase	Importin	NLS in C-terminus	[34]
	GAS41 (glioma-amplified sequence 41)	C-terminal coiled-coil	[35]
	MARs (matrix associated regions)	S/TPXX motifs in N- and C- termini	[17]
	Aurora-A	Thr^{1804}	[9]
	P53	NA	[36]
	SNF2h	NA	[37]
Mitosis	Dynein/dynactin	NA	[11]
	Microtubules	Residues 1 900-1 971 in C-terminus	[15]
	Rae1	Residues 325-829 in N-terminus of coiled-coil	[18]
	Emi1	Binds NuMA/dynein complex	[38]
	LGN	Residues 1 878-1 910 in C-terminus	[15,39]
	PARP-5a	Coiled-coil	[31]
	PARP3	Coiled-coil	[40]
	ACRBP	NA	[41]
	Kinesin Eg5	N-terminus and C-terminus	[33]
	Nup188	NA	[42]
	CDK1	Thr^{2055}	[43]
	SUMO-1	Lys^{1766}	[44]
	Rb	LSCEE sequences in C-terminus (1 727-1 731)	[45]
	Tankyrase	NA	[29]
	BRISC	NA	[46]
	Astrin	NA	[47]
	Katanin p80	NA	[48]
Asymmetric division	LGN[a], GPR1/2[b], Pins[c]	NA	[49-51]
	Dynein	NA	[52]
	ABL1	Tyr^{1774}	[53]
	Aurora-A	Ser^{1969}	[54]
	Dvl 1-3	NA	[55]
	Phosphoinositides	NuMA MBD (1 981~2 060)	[56-57]

a、b、c分别代表人、线虫和果蝇一种NuMA相互作用蛋白的同系物(根据参考文献[6]修改); NA: 未知。

a, b and c represent homologs of one of NuMA's binding paeterners in human, *C. elegans* and *Drosophila* melanogaster respectively (modified from reference [6]); NA: not available.

家族马达蛋白Eg5能够在有丝分裂期在染色体附近与NuMA相互作用并且通过调节NuMA的定位影响纺锤体组装[33]。

4 NuMA的可变剪接体

目前, 对于NuMA蛋白在有丝分裂过程中的功能作用的了解相对透彻, 但对于其可变剪接体的研究还相对较少, 只在数篇文献报道。1993年, Tang等[24]应用特异性的抗NuMA抗体进行免疫印迹实验识别出三个条带, 他们根据分子量大小将这三个条带分别命名为NuMA-l(230 kDa)、NuMA-M(195 kDa)和NuMA-S(194 kDa)。并且, NuMA-L在间期定位于细胞核, 分裂期定位于纺锤体两极; NuMA-M和NuMA-S在有丝分裂间期定位于细胞质, 主要位于中心体区域, 分裂期也定位在纺锤体两极[25]。1994年, Zeng等[58]研究者利用NuMA抗体对连续片段化的NuMA进行免疫荧光、免疫印迹及免疫电镜等功能实验, 研究表明, NuMA可变剪接体是核孔纤丝的组成成分。此外, 也有一些课题组利用多种NuMA特异性抗体进行免疫印迹实验发现, 分子量在180~240 kDa多个条带[5]。本课题组前期利用特异性识别NuMA蛋白的自身免疫病人血清进行免疫印迹实验发现, 该抗体能识别180~240 kDa的3~4个条带[59]。

此外, 我们前期通过UCSC Genome Browser及NCBI等数据库的分析发现, NuMA在哺乳动物细胞中可能含有不同的可变剪切形式。依据*NuMA* mRNA分子量可以将这些可变剪接体分成三类: 长型NuMA(2)、中型NuMA(1)和短型NuMA(3)。这三类

剪接体表达蛋白质的相应分子量为240 kDa、110 kDa及47 kDa。后续研究进一步证实, 长型NuMA包括两个成员, 中型NuMA包括两个成员, 短型NuMA包括三个成员(编码蛋白一样)。长型和中型NuMA亚细胞定位一致, 短型NuMA与长、中型NuMA的亚细胞定位完全不一样(图1), 并且可能执行了完全相反的功能——潜在的肿瘤抑制因子[7]。

5 NuMA1的生物学功能

5.1 NuMA在有丝分裂间期的作用

NuMA蛋白质在间期定位于细胞核, 行使着与胞质微管蛋白不同的功能。早在1974年, Berezney等[60]提出了细胞核基质的概念, 细胞核基质指去掉细胞核膜和染色质后不可溶解的并形成三维网状结构的蛋白成分。这些蛋白质在细胞核内占据一定区域, 并且形成类似于胞质细胞骨架的结构框架。尽管这个概念很有吸引力, 同时进行了近40年的研究, 但是细胞核基质的结构成分目前还不太清楚。由于NuMA存在于高度分化及有丝分裂结束后的细胞核中, 它被认为是细胞核骨架的最具吸引力的潜在结构成分之一[4,61-62]。此外, 对于间期NuMA的功能有如下猜测: (1)NuMA进入细胞核只不过是通过细胞核这个屏障暂时屏蔽它与胞质微管的结合; (2)NuMA可能对于有丝分裂产生的子代细胞基因组重建起着关键的作用; (3)NuMA在高度分化的细胞中可能起着与有丝分裂不同的作用。目前, NuMA作为细胞核骨架成分的观点还是被一些学者所接受。首先, NuMA包含一个巨大的coiled-coil结构域, 很可能形成微丝结构。其次, NuMA通过C-端coiled-coil结构域形成大小为200 nm的同二聚体和高级序列的多臂结构。再次, *NuMA*基因在细胞核里的表达丰度很高, 每个细胞核包含约10^6个拷贝, 并且占据细胞核的大部分体积。最后, NuMA与细胞核的形状相关, 非球形的细胞核中检测不到NuMA表达[62], 并且通过免疫金电镜观察到NuMA在核孔纤丝上有表达。尽管还没有确切的证据表明, NuMA是细胞核基质的组成成分, 但是NuMA已经成为最具吸引力的候选细胞核结构成分。有研究表明, 通过RNAi降低*NuMA1*基因的表达将加速乳腺癌细胞MCF7的细胞核的凋亡降解[63]。此外, 体内和体外实验表明, NuMA能够与细胞核内的剪切因子相互作用[58]。

早期的研究表明, 细胞核运输机制的成分可以借助NuMA参与有丝分裂纺锤体的组装。在分裂间期, 小G蛋白Ran主要负责将RNA和蛋白质转入或转出细胞核。在这个过程中, 输入蛋白(importins)对于靶蛋白核定位信号的识别和结合是入核或出核的前提。TPX2(targeting protein for Xklp2)和NuMA等微管结合蛋白就是通过这个运输机制出入细胞核的[34,64]。另外, 一个细胞核运输机制的成分Rae1被提示也参与细胞有丝分裂的功能。Rae1是一个mRNA输出因子, 能够结合微管和NuMA N-端的coiled-coil结构域, 免疫剔除或者过表达Rae1都将导致纺锤体分裂异常, 形成多纺锤极。过表达或者沉默NuMA的表达能够恢复纺锤体的功能, 提示Rae1和NuMA之间存在负向协同的关系[18]。

5.2 NuMA在有丝分裂分裂期的作用

NuMA在有丝分裂期具有重要的生物学功能。事实上, NuMA在纺锤体起始组装时, 定位在中心体比邻的区域, 并不直接与中心体相结合[65]。从这个层面讲, NuMA对于纺锤体形态的维持包括两个方面: 一是依赖于中心体, 通过与微管直接相互作用把微管运到中心体处起始组装; 二是不依赖于中心体, 只是把微管聚集在纺锤极附近, 维持纺锤极的结构稳定性。目前, 对于NuMA在纺锤极维持其稳定性的功能机制有两个: 第一, NuMA二聚体与动力蛋白(dynein)及微管结合, 并借助动力蛋白的驱动力将微管负端运输到纺锤极处形成一个潜在的结构——“纺锤极基质”; 第二, NuMA-动力蛋白复合物利用其自身的微管结合模体与微管簇瞬时结合并将微管负端聚集在纺锤极。不仅NuMA对纺锤体的组装和稳定起着非常关键的作用, 其磷酸化状态对于细胞进入和离开分裂期也非常重要。G_2晚期, 核膜开始崩解, NuMA被$p34^{cdc2}$高度磷酸化, 然后借助动力蛋白介导的运输机制从细胞核转移到纺锤极, 直到有丝分裂后期, 由于Cdk1(cyclin-dependent kinase 1)表达量的下降, NuMA发生去磷酸化, 并离开纺锤极。NuMA四个磷酸化位点是协同作用的, 缺一不可, 任一位点发生突变都将导致NuMA无法定位在纺锤极, 而是定位到质膜[16]。另外, 在分裂期结束前, 有丝分裂后期促进复合物(anaphase-promoting complex, APC)的抑制蛋白Emi1[也成为FBXO5(F-Box protein 5)]在纺锤极处于NuMA及动力蛋白形成一个复合物, 其功能被屏蔽[38,66-67]。细胞进入有丝分裂后期后, 由于cyclinB的降解及Cdk1抑制蛋白的重新表达,

Cdk1开始失活, NuMA发生去磷酸化, 并与动力蛋白及Emi1分开[68]。解离后的Emi1进而抑制APC/C复合物的功能, 使细胞离开分裂期。

5.3 NuMA在不对称分裂中的作用

NuMA在不对称分裂过程中也起着非常重要的作用。在不对称分裂过程中, 一部分NuMA被偏好性地招募到细胞皮层(cell cortex)的某一部分区域, 介导纺锤体的锚定。这种偏好性招募的产物将被最靠近纺锤极的星状微管捕获进而产生不对称的纺锤体定位[8,69-70]。前期研究表明, 在线虫和果蝇中, NuMA同源蛋白Lin-5、Mud与受体依赖的三体G蛋白(Gα)和其调节蛋白(GPR-1/2/Pins)形成复合物(LIN-5/GPR-1/2/Gα和Mud/Pins/Gα), 然后被招募到细胞皮层调节纺锤体不对称定位[71]。破坏G蛋白或者其调节蛋白都将导致染色体排列异常, 提示Gα和其调节蛋白是不对称细胞分裂纺锤体定位信号通路中的关键组成成分。后续研究表明, 在人类中, NuMA能与Gα及Gα调节蛋白LGN形成复合物(NuMA/LGN/Gα), 调节纺锤体不对称定位[6]。

在有丝分裂间期, LGN定位于细胞质, 当细胞进入分裂期, LGN通过与NuMA C-端一段结构域相互作用定位到纺锤极。LGN还可以通过与质膜锚定蛋白Gα亚基的相互作用富集在细胞皮层[39,72]。事实上, LGN与NuMA相结合的区域同微管与NuMA结合的区域正好重合, LGN与微管竞争性结合NuMA, 控制微管的聚合和解聚。LGN与NuMA的结合依赖于NuMA磷酸化状态, 如果加入蛋白激酶抑制剂星形孢菌素, 将阻止LGN与NuMA相结合[73]。此外, LGN与NuMA结合能够释放一部分NuMA分子参与不对称纺锤体的定位。并且, LGN可以通过自身N-端和C-端发夹样结构进行自连, 从而抑制自身的表达。这种抑制效应可以被Gα和NuMA与它N-端和C-端结构域的结合而消除, 并且这种双重相互作用, 能够介导NuMA转运到质膜。基于现有NuMA和LGN的研究结果, 很可能存在这样一种作用机制, 特异性改变NuMA一个或者几个磷酸化位点, 将影响NuMA与LGN的相互作用关系, 进而影响NuMA定位到细胞皮层, LGN成为了NuMA在有丝分裂过程和不对称分裂过程行使功能的分子开关。尽管如此, 对于纺锤体锚定在质膜的作用机制还是不太清楚。不过有研究报道, 在线虫里面, 动力蛋白被证实作为一种重要的蛋白因子提供一种皮层驱动力作用于星状微管[52]。结合以前的研究, 在线虫中可能存在这样一种机制, NuMA同源蛋白Lin-5与GPR-1/2(G protein-coupled receptor-1/2)和Gα形成复合物并定位到质膜, 质膜借助动力蛋白依赖的作用机制将一个纺锤极沿着皮层的方向拉拽进而形成不对称的纺锤极定位。尽管此作用机制还没有在果蝇和人类中得到证实, 但是因为这些关键调控蛋白的保守性, 在人类和果蝇中, NuMA和Mud也可能是通过这种作用机制在有丝分裂和不对称分裂过程中调节纺锤极的定位。

5.4 NuMA在人类疾病中的作用

早期对NuMA蛋白质的研究表明, NuMA是一些自身免疫病人的自身抗原[74-75], 如系统性红疤狼疮(systemic lupus erythematosus, SLE)、风湿性关节炎(rheumatic arthritis, RA)等。后续研究报道, NuMA过表达会诱导多极纺锤体形成, 导致细胞分裂异常, 因此认为与肿瘤发生相关。如*NuMA*基因定位于11q13区域, 在急性早幼粒细胞白血病里, NuMA与视黄酸受体α形成融合基因, 并表达融合蛋白NuMA-RARA(retinoic acid receptor alpha), 这个融合蛋白能够与类X受体α相互作用, 并共同作用于转基因小鼠白血病细胞的发育过程[30]。NuMA第794位氨基酸发生错义突变A794G将会显著增加患乳腺癌的风险[76]。PCR和Sourthern blot结果显示, 在神经胶质瘤细胞U-251中存在NuMA多个转录本[24]。急性红斑脑炎与能识别NuMA的血清紧密相关[77]。Briggman等[78]通过对86例结肠癌患者血清、72例患普通胃肠病患者血清、80例有患食管癌风险的患者血清及141例年龄相匹配的健康人血清中NuMA水平进行分析, 以期将NuMA作为结肠癌早期诊断的肿瘤标志物。研究结果表明, NuMA对于食管癌患者的敏感性远高于另一诊断标志物CEA(carcino-embryonic antigen)。Hasholzner等[79]分析了507例食管癌患者血清和418例健康人的血清NuMA水平, 结果表明, 其不具备临床相关性。人源*ASPM*[abnormal spindle microtubule assembly, 也称为*MCPH5*(microcephalin 5)]基因功能丧失可以导致常染色体隐性头小畸形疾病[80], 具体致病机理还不是很清楚, 但是在线虫中, ASPM-1可以结合NuMA同源蛋白Lin-5并将它招募到减数分裂的纺锤极附近控制纺锤体的定位[81], 由此可以推测, 在人类和小鼠里, 主要通过招募NuMA到中心体进而控制纺锤体的定位[82]。最新研究表明, NuMA1[也

称为NMP22(nuclear matrix protein 22)]在膀胱癌等多种肿瘤高表达, 并且NuMA1可作为膀胱癌的肿瘤标志物[83-85]。

5.5 NuMA在核移植中的作用

NuMA蛋白质近些年在胚胎移植方面的应用得到快速发展。自1997年首例体细胞克隆绵羊多莉出生至今, 哺乳动物核移植技术已经发展十余年, 随后也在数个物种中获得了成功, 但核移植效率低, 并且克隆个体出生率只有1%~5%。在细胞核移植过程中, NuMA的分布对核移植成功率很关键[86-88]。另有研究表明, 在非人灵长类体细胞核移植胚胎中一般都会出现*NuMA*缺失、驱动蛋白Eg5分布紊乱及纺锤体形态异常等现象[89-90]。但也有研究报道, NuMA在大部分猴子和人类体细胞核移植胚胎中正常表达, 并且纺锤体也正常组装, 间接否认NuMA丢失影响核移植效率的观点[20,91]。此外, NuMA可能在基因重组中也发挥作用, 而核移植胚胎发育过程中是否存在NuMA对基因重组的影响尚无相关报道[92]。

6 结语与展望

自NuMA被发现至今已有30余年, 其功能涉及到生命活动的方方面面, 如细胞周期调控、有丝分裂、不对称分裂、细胞凋亡、胚胎移植和肿瘤发生发展等, 但人类对NuMA具体作用机制的认识尚不够深入, 尤其对于NuMA在间期细胞核内的作用、在生殖细胞减数分裂、细胞核形成及结构维持、可变剪接体的分类及功能等方面有待进一步研究。对NuMA的深入探索有利于更加全面了解NuMA的功能, 尤其对于胚胎移植、疾病及肿瘤关系研究, 将进一步解决核移植效率相关问题, 推动核移植技术的发展, 促进人类治疗性克隆临床应用的步伐, 并对于人类疾病和肿瘤的诊治提供新策略和方向。

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