综 述

细丝蛋白A在细胞黏附和迁移中的作用

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摘要 细胞迁移在发育、伤口愈合、炎症反应和肿瘤转移等多种病理生理过程中发挥重要作用。细丝蛋白A(filamin A, FlnA)是一种在各组织细胞中广泛表达的微丝结合蛋白, 其表达异常导致细胞迁移功能障碍。该文回顾了相关的文献, 首先介绍生理情况下细丝蛋白A的功能, 接着介绍细丝蛋白A基因突变和表达异常导致的多种遗传性疾病及其与肿瘤转移的关系, 突出细丝蛋白A对迁移的影响在这些疾病发病中的作用, 最后深入探讨了细丝蛋白A影响细胞迁移和黏附的可能机制。

关键词 微丝; 细胞黏附; 细胞迁移; 细胞膜结构

Role of Filamin A in Cell Adhesion and Migraion

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Abstract Cell migration is essential for development, tissue remodeling and wound healing, and is abnormal in many pathological states. Filamin A is an actin-binding protein and is widely expressed in various cell types. Mutations in filamin A gene are the cause of a wide range of genetic diseases, while abnormality in filamin A expression is resposible for cancer metastasis. Filamin A regulates cytoskeleton rearragement and palys a pivotal role in cell shape determination. Depletion of filamin A leads to obvious cell migration defects. In this review, we discuss the implication of filamin A in genetic diseases and cancer metastasis, emphasizing the impact of cell migration defects on these diseases. Further more, we discuss the role of filamin A on cell migration and its underlying mechanism.

Key words actin cytoskeleton; cell adhesion; cell migration; cell membrane structure

细丝蛋白A(filamin A, FlnA)是非肌性肌动蛋白结合蛋白, 由位于X染色体上的基因*FLNA*编码, 分子量约280 kDa, 表达广泛[1]。如图1A所示, FlnA由两个同源亚基非共价结合而成, 每个亚基又包含一个氨基端肌动蛋白结合结构域(actin binding domain, ABD)和24个串联的重复片段(filamin β-sheet repeats, FR), 中间间隔两个铰链结构(hinge region) H1、H2。H1、H2将24个FR分 隔 为Rod1(FR1-15)、

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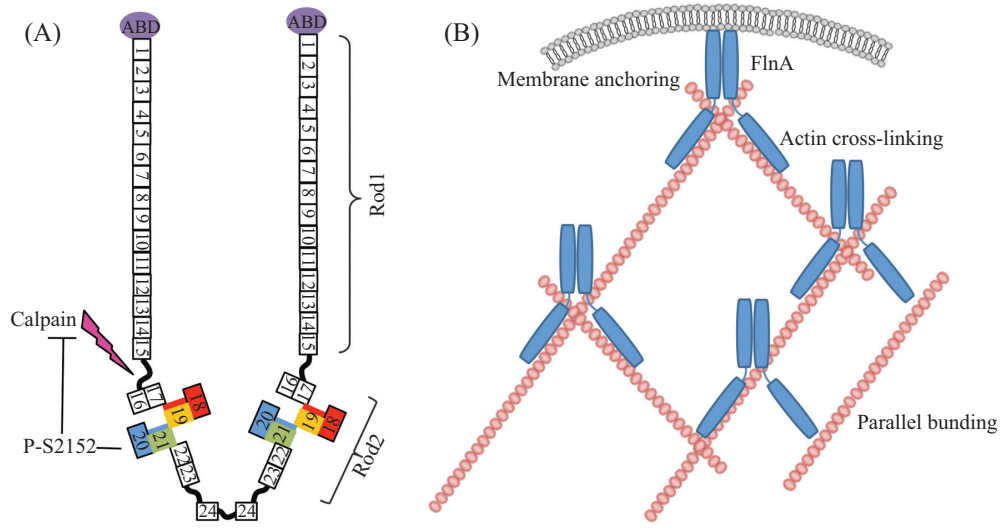
Rod2(FR16-23)以及聚合结构域FR24(dimerization domain)三部分[2]。FlnA能将微丝相互交联成网状或捆绑成束状(图1B)[3], 此外, 还通过其众多的FR与不同分子结合, 介导多种不同的功能(表1)。本文回顾了相关文献, 首先介绍生理情况下FlnA的功能, 接着介绍*FLNA*突变和表达异常导致的多种遗传性疾病及FlnA与肿瘤转移的关系, 突出FlnA对迁移的影响在这些疾病发病中的作用, 最后深入探讨了FlnA影响细胞迁移和黏附的可能机制。

1　FlnA与细胞骨架重排及细胞形态的关系

无论是生理还是病理情况下, 细胞都需要不断改变其形态或机械特性来适应周围环境。如白细胞吞噬异物的过程、血管内皮细胞对血流切应力的反应、肿瘤转移的过程等。与植物细胞不同, 动物细胞没有细胞壁。然而, 动物细胞内有由微丝、微管和中间丝组成的细胞骨架系统, 该系统为细胞提供机械强度并维持细胞形态; 同时, 当受到外界信号刺激时, 能进行细胞骨架重排, 改变细胞形态以适应环境的变化。这样, 细胞既能抵抗外界机械应力的作用, 又具有一定的可塑性和适应能力。FlnA能与肌动蛋白结合, 其结构特点使其在调控细胞骨架的机械强度及可塑性方面起到了重要作用。FlnA是由两个亚基组成的二聚体, 除了氨基端的ABD, 其Rod1上还有第二处ABD, 这样FlnA上就有多个ABD, 另外每个蛋白亚基上都具有两个可以活动的铰链结构(H1、H2), 从而既赋予了FlnA对微丝的高亲和力, 又使其结构具有一定的灵活性[3-4]。FlnA的这些结构特点使其能够改善细胞骨架的机械特性, 使细胞骨架不会轻易在外力的作用下变形, 从而细胞能够承受更多的机械应力[2,5]。

细胞的形态特征取决于细胞骨架的具体组装形式, 并且通过细胞骨架与细胞膜的连接, 将这些组装形式反映到细胞的外部形态上[6]。当细胞膜与网状的扁平微丝骨架相连时, 细胞可以表现为细胞周边的片状突起; 而当细胞膜与平行排列成束状的微丝骨架相连时, 则可表现为指状、针状的突起。FlnA能结合多种细胞膜蛋白及信号蛋白(表1), 将微



A: FlnA的结构示意图。FlnA由两个同源亚基非共价结合而成, 每个蛋白亚基又包含一个氨基端ABD和24个FR, 两个铰链结构将这些FR分为Rod1(FR1-15), Rod2(FR16-23)以及聚合结构域(FR24)。Rod2上奇数号FR(19、21)上的蛋白结合位点被偶数号FR(18、20)所遮蔽, 从而使Rod2的结构较Rod1更为紧凑; FlnA肽链上第2152位丝氨酸可被多种蛋白激酶磷酸化, S2152磷酸化能抑制calpain对FlnA的水解; B: FlnA可以将微丝以相互垂直的角度交联成网状或者平行捆绑成束, 并将微丝细胞骨架与细胞膜相连, 从而影响细胞的形态和结构。

A: schematic representation of the structure of FlnA. FlnA is a non-covalent dimer composed of an N-terminal actin-binding domain (ABD) followed by 24 β sheet repeats. Two flexible hinge regions separate the repeats into Rod1 (repeats 1-15), Rod2 (repeats 16-23) and the dimerization domain (repeat 24). The eight repeats in the Rod2 domain form a structure that is far more compact than those of rod 1 segments containing equivalent numbers of FlnA repeats. Pairing of even-numbered repeats 18 and 20 with their neighboring repeats 19 and 21 conceal the partner binding pocket in repeats 18 and 20. Susceptibility of FlnA to calpain cleavage is regulated by its phosphorylation. Specific phosphorylation at S2152 in the 20th repeat is of particular importance because it can protect FlnA against cleavage; B: FlnA anchors the actin filamins at the membrane and crosslinks them into orthogonal networks or parallel bundles.

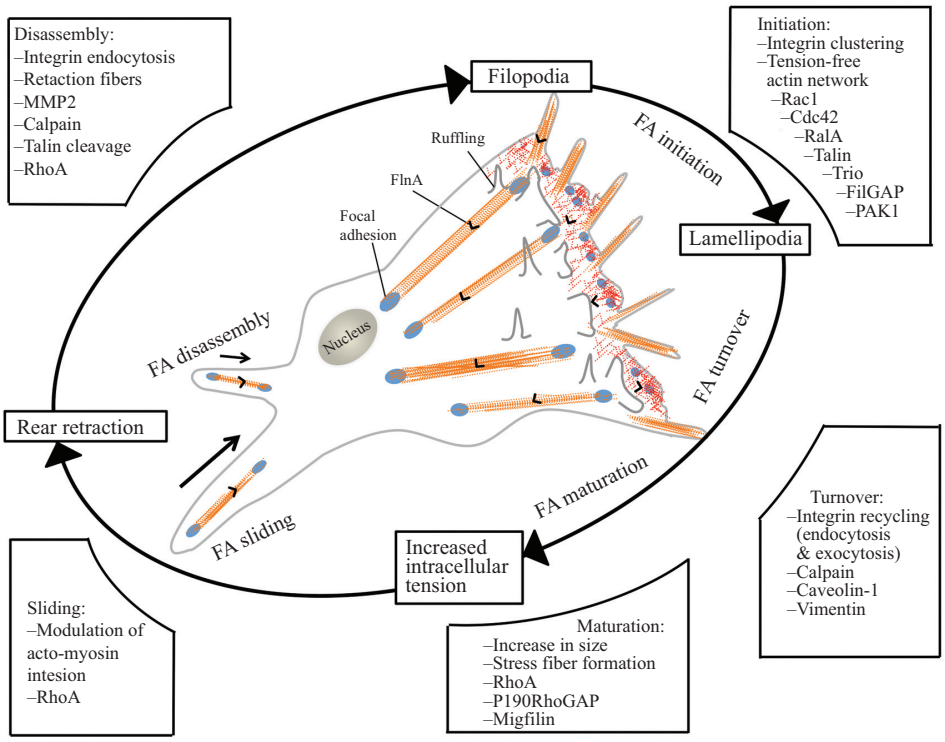
图1　细丝蛋白A的结构及其对细胞骨架的作用(根据参考文献[3]修改)

Fig.1　Schematic representation of the structure of FlnA and its effect on actin cytoskeleton (modified from reference [3])

表1 FlnA结合蛋白

Table 1 FlnA binding partners

FlnA结合蛋白 FlnA binding partners	结合位点 Binding sites	相互作用的意义 Significance
F-actin	ABD, rod-1	FlnA crosslinks actin filaments with unique mechanical and physiological properties[1,15]
Calmodulin	ABD	Regulates F-actin binding *in vitro*[16]
R-Ras	3	Enhances integrin activation and maintains endothelial barrier[17-18]
Syk	5	FlnA is required for itam-mediated receptor signaling in platelet[19]
Vimentin	1-8	FlnA regulates β1integrin cell surface expression and activation via its association with vimentin and PKCε[20-22]
Supervillin	8-10, 20-22	Cell spreading[23]
Pro-Prion	10, 16-18, 20-21, 23	Enhances the binding of filamin-A with β1 integrin, and promotes cell spreading and migration in melanoma[24-25]
Androgen receptor	16-19	Required for androgen-induced cell migration[26-27]
GPI bα (CD 42b)	17	Intracellular trafficking and maintains the size of platelets[28-29]
Dopamine D2 and D3 receptors	19	Stabilizes β-arrestins-filamin-A complex[30-31]
Migfilin (FBLP-1)	21	Migfilin disconnects FlnA from integrin and promotes talin-integrin binding Interaction between FlnA and migfilin connects cell-matrix adhesion to the actin cytoskeleton[32-35]
Integrin β	21	Adhesion FlnA competes with talin for integrin cytoplasmic binding site and inhibit integrin activation[36-37]
Tissue factor	22-24	Supports cell spreading and migration[38]
Caveolin-1	22-24	Integrin intracellular trafficking[39-40]
CEACAM 1	23-24	Reduces cell migration[41]
Rho/Rac/Cdc42	21-24	Remodeling of cytoskeleton[1]
FilGAP	23	Mechanoprotection Rho- and ROCK-regulated GAP for Rac. FlnA-binding is required for cell spreading and stimulates GAP activity[14,42]
Trio	23-24	GEF for RhoG/Rac1 and RhoA Required for ruffling[10]
RalA	24	Filopodia formation[8]
p190RhoGAP	?	Expression of calpain-insensitive FlnA excludes p190RhoGAP from the lipid raft, thereby increase Rho activity[43]
ROCK	24	Remodeling of cytoskeleton[1,44]
LL5β	ABD, 24	LL5β directs the translocation of FlnA and its associated SHIP2 to sites of PtdIns(3,4,5)P_3 accumulation and lamellipodium formation[11]
SHIP-2	?	Cell adhesion, submembrane actin remodelling[45]
RefilinB	15-24	Stabilizes perinuclear actin actin networks and regulates nuclear shape[46]
BRCA1	23-24	Facilitates the recruitment of BRAC1 and RAD51 to DNA damage sites and stabilizes the DNA-PK holoenzyme[47]
BRCA2	21-24	Required for efficient homologous recombination DNA repair and recovery of G_2/M phase arrest[48-49]



细胞迁移伴随着黏着斑的动态改变, FlnA能影响迁移过程中动态变化的黏着斑。图正中是正在迁移的细胞的示意图, 在其前端可以看到突起的片状伪足、丝状伪足和membrane ruffling及其下方的黏着斑。在迁移的过程中, 细胞前端不断生成黏着斑, 随着细胞前移, 新生成的黏着斑有的turnover, 有的则在细胞内张力的作用下成熟; 在细胞后方, 黏着斑或者分解或者sliding, 引起细胞收缩, 推动细胞向前方移动。FlnA对这些变化的黏着斑作用的可能的机制, 包括FlnA可能影响的结构及相应的分子, 标在附近相应的方框内。

Cell migration can be viewed as a cycle of dynamic focal adhesion remodeling. This schematic representation of a migrating cell highlights filopodia, lamellipodia, retraction fibers and different types and states of focal adhesions. FlnA regulates the dynamic remodeling of focal adhesion. An text box adjacent to each different focal adhension state describes the possible mechanism by which FlnA regulates them.

图2　细丝蛋白A对细胞黏附和迁移影响的可能机制

Fig.2　Possible mechanism of the effect of FlnA on cell adhesion and migration

丝骨架与细胞膜连接在一起, 并通过与其结合的各种信号蛋白, 调控细胞骨架的重排和细胞形态。如FlnA作为支架蛋白参与RalA诱导的丝状伪足, 及Trio、PAK1(p21-activated kinase1)诱导的membrane ruffling的产生(图2)[7-10]。目前一般认为, membrane ruffling来源于未能与细胞外基质黏附而翻向细胞背侧的片状伪足[11-12]。又如Rho GTPase家族是调控细胞骨架重排的重要分子[11]。FlnA能与FilGAP结合并调节其活性, 后者抑制片状伪足的生成, 促进细胞周边blebbing的产生[14]。

2　*FLNA*突变与遗传病的关系

*FLNA*功能的缺陷可导致包括脑、骨骼、心脏在内的多种发育畸形[50-51]。脑室周围结节状灰质异位(periventricular nodular heterotopia, PNH)是一种脑发育畸形, 其发病是由于在胚胎发育过程中, 神经元不能向大脑皮质迁移, 导致神经核团在脑室附近的异位, 典型症状可有癫痫发作。PNH的发生由*FLNA*突变引起, 这些突变常导致mRNA的剪接异常或截短蛋白的产生, 也可以是错义突变, 突变的结果是细胞内FlnA功能的缺失[1,52-54]。FlnA功能缺失导致神经元细胞骨架重塑障碍, 神经元与胶质细胞黏附异常, 从而神经元不能从脑室周围迁移到大脑皮层的正常部位[54]。PNH主要见于女性杂合子, 这些患者几乎没有男性后代, 并且表现为过度的流产, 提示X染色体显性遗传所致的男性半合子的胚胎期死亡[51]。PNH患者除了神经系统的异常, 还可以表现为心脏瓣膜异常、小关节过度伸展、动脉导管未闭等, 提示FlnA在结缔组织和心血管系统中的功能[51]。*FLNA*突变还可见于耳–腭–指综合征(oto-palatodigital syndrome, OPD)、额骨干骺端结构不良(frontometaphyseal dysplasia, FMD)、梅–尼二氏综合征(Melnick-Needles syndrome, MNS)等多种先天性畸形[54-55]。另外, *FLNA*的错义突变可能是家族性

心脏瓣膜营养不良的病因[2]。

3 FlnA与肿瘤转移的关系

FlnA过表达可见于多种类型肿瘤, 包括前列腺肿瘤、乳腺癌、肺癌、结肠癌、黑色素瘤、成神经细胞瘤、鳞状上皮细胞癌、胆管癌等[56-61]; 其基因突变也可见于乳腺癌和结肠癌[62]。FlnA参与肿瘤的发生、血管新生和转移[3,58,63]。目前, FlnA在肿瘤转移中的作用尚存在争议, 其既可以促进肿瘤转移也可以抑制肿瘤转移[3,7]。肿瘤的转移是一个复杂的过程, 涉及肿瘤细胞从原发部位脱离、降解细胞外基质、迁移、渗入血管、随血液循环系统转移并在其中存活、移出血管、在新的部位定居并增殖等多个步骤。一方面, FlnA能促进肿瘤转移。在肿瘤转移的过程中, 细胞需要不断改变其形态, 抵抗机械应力, 并具有很强的运动能力及适当的黏附能力以定居于新部位。FlnA与微丝相互作用形成的质膜下细胞骨架, 为细胞提供机械强度并支持细胞形态改变, 因而能促进肿瘤的转移。FlnA表达增高与肝细胞癌和乳腺癌的恶性程度相关[57,64]。用shRNA(short hairpin RNA)敲除*FLNA*能抑制乳腺癌细胞和黑色素瘤细胞迁移, 抑制裸鼠皮下接种的黑色素瘤细胞的远处转移[65]。另一方面, FlnA也能抑制肿瘤转移。乳腺癌患者癌组织内FlnA表达降低与癌细胞淋巴结转移相关, 沉默*FLNA*会影响乳腺癌细胞(表达ErbB2)与基质的黏附, 抑制细胞迁移[66]。敲除*FLNA*能促进基质金属蛋白酶活性, 增强纤维肉瘤细胞的侵袭性[67]。

针对这种矛盾现象, 最近有文献提出假说, 该假说认为, FlnA在细胞内的位置决定了其对肿瘤的作用, 即当FlnA位于胞质时, 能促进肿瘤的转移; 而当FlnA被钙蛋白酶(calpain)水解, 生成的水解片段入核时, 则能通过调控基因转录等方式抑制肿瘤的转移[3]。该假说得到了相关文献的支持。FlnA的水解能抑制前列腺癌的转移, 而细胞质内完整的FlnA则促进前列腺癌的转移[61]。雄激素在前列腺癌的发生发展中起重要作用, 临床上可采用雄激素剥夺疗法治疗前列腺癌。在受到雄激素刺激时, 雄激素受体(androgen receptor, AR)与胞质内的FlnA相互作用形成FlnA/AR复合物, 该复合物能激活FAK、Rac以及相应的信号通路, 促进细胞迁移[26]。除了以复合体的形式激活迁移相关的信号通路, FlnA与AR的结合还能促进AR进入细胞核, 启动基因转录, 进而促进前列腺癌转移[27]。然而, 在calpain的作用下, FlnA水解产生的90 kDa的片段进入细胞核时, 则能抑制AR相关性基因的转录, 并抑制肿瘤转移[68]。对于激素难治性前列腺癌(hormone refractroy prostate cancer, HRPC), 肿瘤细胞的生长、转移不依赖雄激素的刺激。在HRPC细胞系C4-2中, FlnA水解受抑制, 转染编码FlnA入核片段的质粒则能重建其对康士得(Casodex, 抗雄激素药物)的敏感性, 从而提示在HRPC中, FlnA水解对肿瘤的保护性作用消失, 肿瘤无需依赖雄激素刺激就可以得到进展[69]。

目前, 有关FlnA在细胞内的位置对肿瘤转移的影响方面的文献尚较少, 大部分研究集中在*FLNA*敲除或者过表达对肿瘤的影响。另外, 肿瘤的转移是一个非常复杂的过程, 涉及肿瘤细胞的迁移、肿瘤细胞间以及细胞与基质的黏附的改变等多方面。在肿瘤转移的不同阶段, 对肿瘤细胞的迁移、黏附能力的要求也不尽相同。FlnA蛋白肽链2 152位点上丝氨酸(Ser2152)的磷酸化能抑制calpain对FlnA的水解[2,70-71]。然而, 有研究表明, FlnA的磷酸化既能促进细胞迁移也能抑制细胞迁移[36,72]。在原代黑色素瘤细胞内, Wnt5A/ROR2信号通路的激活能促进calpain对FlnA的水解, 进而促进黑色素瘤细胞的迁移[73]。因此, 上述假说还有待进一步的验证。该假说为我们提供了一个很好的认识FlnA的角度。除了*FLNA*敲除和过表达对肿瘤转移的作用, 我们还要考虑FlnA水解、其转录后修饰的影响以及FlnA的水解片段进入细胞核后如何发挥作用等。值得注意的是, 无论是在胞质内还是在细胞核内, FlnA作为一个支架蛋白, 都需要通过与其他蛋白的相互作用来调控各种细胞功能, 我们在考虑FlnA对细胞功能的影响时, 可能还需要考虑其结合蛋白的功能、具体的细胞类型及细胞外刺激以及FlnA具体影响了肿瘤转移的哪个方面等内容。

4 FlnA与其结合蛋白间相互作用的调控

FlnA与其结合蛋白间的相互作用主要通过以下几种方式进行调控: (1)构象改变。FlnA可以作为细胞内机械张力的感受器。在机械张力的作用下, *FLNA*敲除的细胞较正常细胞更容易发生凋亡[74]。此外, FlnA还参与机械门控离子通道对机械刺激的反应[75]。FlnA上的Rod2包含FR16-23, 这些FR相互作用, 其中奇数号FR(19、21)上的蛋白结合位点被偶数

号FR(18、20)所遮蔽, 这也使得Rod2的结构较Rod1更为紧凑(图1A)[76-77]。FlnA通过其氨基端ABD与微丝细胞骨架结合, 感受细胞内的机械张力的改变, 在机械张力的作用下, Rod2上FR间的相互作用发生改变, 被遮蔽的蛋白结合位点得以暴露[78-79]。机械张力的刺激还能改变FlnA对不同蛋白的亲和力, 如对整合素的亲和力增加, 而对FilGAP的亲和力降低[80]。(2)磷酸化修饰。FlnA上的数个位点能够被多种蛋白激酶磷酸化, 并且FlnA的磷酸化修饰影响其对蛋白的结合能力及细胞功能[9,71,81-84]。其中, 对Ser2152磷酸化的研究较多, Ser2152的磷酸化能抑制calpain对FlnA的水解(图1A)[2,70-71]。目前, Ser2152磷酸化是否促进FlnA对整合素的结合尚存在争议[78-79,85]。此外, 对FlnA结合蛋白的磷酸化也能调控其与FlnA的亲和力。整合素β亚基胞浆段Thr758的磷酸化能抑制其对FlnA的结合, 促进其对talin的结合[86]。(3)蛋白的水解作用。如前文所述, FlnA被calpain水解产生的片段可以进入细胞核, 在细胞核内通过与转录因子等的相互作用, 发挥与其在胞质内不同的作用。另外, FlnA的水解能调控p190RhoGAP的活性, 进而影响细胞骨架重排和细胞形态[43]。(4)竞争性结合作用。如表1所示, 不同的蛋白可以与FlnA上相同的位点结合, 从而相互竞争。Migfilin与整合素对FlnA的竞争结合参与调控细胞与基质的黏附和迁移[32,34-35]。(5)整合素clustering。FlnA上有多处整合素结合位点, 并且这些位点可以同时结合整合素, 从而可以对整合素产生clustering的作用, 促进黏着斑的形成和对基质的黏附[2,87]。

5　FlnA对迁移的影响及其可能机制

正如前文所述, FlnA在肿瘤转移的过程中起到了重要作用。肿瘤转移包含细胞迁移能力的改变, 因此, FlnA在肿瘤转移过程中的两面性也反映出其对细胞迁移的影响。FlnA可以促进迁移, 敲除*FLNA*能促进黑色素细胞和乳腺癌细胞的迁移[18,65,88]。在NIH3T3和HT1080细胞中, 雄激素的刺激能促进FlnA与AR的结合, 进而激活整合素β1和FAK, 促进细胞迁移[26]。然而, FlnA也可以抑制细胞迁移。在中国仓鼠卵巢细胞中, FlnA对整合素的过度紧密结合抑制细胞表面突起的生成和细胞迁移[89]。当在细胞内共同表达CEACAM1-L和FlnA时, 能减少RalA的激活, 影响黏着斑, 抑制细胞的迁移[41]。

迁移是一个高度复杂的过程(图2)。在细胞迁移的过程中, 首先, 在细胞的运动前缘伸出突起, 包括板状伪足和丝状伪足; 其次, 突起与细胞外基质之间形成新的锚定位点, 使突起固定在基质表面; 然后以附着点为支点向前移动, 同时细胞后缘的附着点与基质脱离使细胞的尾部前移[90]。在这个过程中, 细胞不停地与基质发生相互作用, 不断地进行细胞骨架重排, 产生片状伪足、丝状伪足、应力纤维和黏着斑等结构, 这些结构也不断的进行着动态变化。FlnA通过影响上述细胞结构影响细胞的迁移。FlnA参与RalA诱导的丝状伪足及Trio GEFD1、PAK1诱导的membrane ruffling的产生[8-11,91]; FlnA能与FilGAP结合并调节其活性, 后者抑制片状伪足的生成, 促进细胞周边blebbing的产生, 进而促进肿瘤细胞的阿米巴式迁移(amoeboid migration)[14,92]。在乳腺癌细胞内, 当用shRNA沉默FlnA时, 能导致细胞前缘黏着斑的分解并促进细胞迁移[66]。同时敲除小鼠胚胎成纤维细胞内的*FLNA*、*FLNB*能抑制黏着斑的成熟, 抑制细胞对基质的黏附, 减小黏着斑体积, 降低应力纤维的数量[93]。RSK2(p90 ribosomal S6 kinase 2)通过促进FlnA的磷酸化, 抑制黏着斑的成熟和应力纤维的生成, 进而促进细胞迁移[36]。

FlnA通过其不同的结合蛋白, 参与不同的调控细胞迁移的信号通路(表1)。首先, FlnA通过其与小GTPase家族成员R-Ras的作用促进细胞迁移。R-Ras与FlnA的FR3结合, 敲除*FR3*能抑制两者结合, 抑制细胞对基质的作用和细胞迁移[18]。其次, FlnA可以通过刺激c-Met转录影响迁移。c-Met是肝细胞生长因子(hepatocyte growth factor, HGF)的唯一受体, 在细胞的生长、迁移过程中发挥极其重要的作用, 具有很强的致癌作用。经γ射线照射后的成纤维细胞, 其细胞内c-Met和FlnA的表达水平升高, 并且这些射线照射后的细胞能促进其周围鳞状细胞癌细胞的增殖和迁移[59]。当肿瘤细胞内FlnA表达缺乏时, 细胞内c-Met的表达也降低, 在受到HGF的刺激时, 细胞的迁移能力也降低[94]。进一步的研究发现, FlnA通过与Smad2相互作用, 促进c-Met转录, 进而促进HGF/c-Met/Akt信号通路的传导, 使细胞迁移能力增强。除了影响R-Ras和c-Met介导的信号通路, 如前文所提到的, FlnA还参与RhoGTPase家族成员(如Rac、PAK1、Cdc42、RalA、RhoA、Trio和FilGAP等)对细胞骨架的重排并在细胞迁移的过

程中发挥作用[8-11,14,91-92]。此外, 在肾小球系膜细胞受到IGFBP-5(insulin-like growth factor-binding protein-5)刺激后, FlnA去磷酸化并被calpain降解, 生成的FlnA的羧基端水解片段能促进Smad3/4入核, 后者促进层黏连蛋白的转录, 最终导致细胞迁移的增强[95-96]。

综上所述, 由于FlnA其可以调节多条信号通路, 影响多种细胞结构, 这也就解释了其在不同类型的细胞和不同的细胞外刺激下, 对细胞迁移可能产生的截然不同的效应。

6 FlnA对细胞与基质黏附的影响

细胞与基质的黏附在细胞迁移的过程中起到了至关重要的作用。细胞膜上的黏附受体, 通过各种结构和信号蛋白, 将细胞外基质与细胞骨架连接在一起, 形成成分复杂的黏着斑。伴随着细胞的位移, 黏着斑不断循环往复于产生(initiation)、成熟(maturation)和分解(disassembly)的过程中, 其形态和成分也在不断地发生变化[97]。如图2所示, FlnA影响迁移过程中动态变化的黏着斑[18,36,41,66]。

6.1 FlnA调控整合素的激活和黏着斑的形态

整合素是细胞主要的黏附分子, FlnA能调控整合素的激活。Talin与β整合素结合是激活整合素(inside-out activation)的主要途径, 并且talin是介导整合素与细胞骨架相连的主要蛋白之一[86]。FlnA与β整合素的结合位点与talin部分重叠, 竞争talin对整合素的结合, 从而抑制整合素的激活[37]。在整合素激活后, FlnA与talin的竞争还能进一步调控黏着斑的生成和形态。Talin通过对整合素的结合, 竞争置换出FlnA, 不再与整合素结合的FlnA转而与FilGAP结合, 抑制后者对Rac的灭活, 进而促进黏着斑和应力纤维的生成, 促进细胞在基质表面的铺展(outside-in signaling)[98]。对FlnA和整合素的磷酸化修饰能调节两者的结合[79,86]。RSK2能抑制整合素的激活, 促进FlnA的磷酸化和其对整合素的结合能力, 促进细胞迁移[36]。该研究认为, RSK2可能通过对FlnA的磷酸化, 增强后者结合并抑制整合素的能力。然而, 值得注意的是, 当细胞过表达显性激活型RSK2(dominant active RSK2, DA-RSK2)时, talin对整合素的结合也增加, 细胞内成熟的黏着斑和粗大的应力纤维的数量则减少。我们认为, RSK2激活后, 通过不同的信号途径分别作用于talin和FlnA, 两者对整合素的亲和力的最终平衡, 决定了整合素的激活情况及黏着斑的生成和具体形态。

此外, migfilin也通过其与FlnA的相互作用, 参与调控整合素的激活和黏着斑的生成。当用siRNA干扰细胞内migfilin的表达时, 能抑制黏着斑的产生, 抑制细胞在基质表面的铺展和细胞迁移[32,34-35]。进一步的研究表明, migfilin与整合素竞争FlnA的结合位点, 抑制FlnA对整合素的结合, 从而起到激活整合素的作用[33-34]。然而, migfilin基因的敲除并不影响小鼠的正常发育和表型, 来源于migfilin基因敲除小鼠的成纤维细胞和角质细胞也没有明显的黏附和迁移功能异常[99], 或者只表现为骨重建的障碍[35], 从而使migfilin在调控整合素激活及细胞黏附方面更普遍的生物学意义受到质疑。

6.2 FlnA调控黏着斑的turnover

除了在整合素的激活以及黏着斑的产生和形态方面起到调控作用, FlnA还能调控黏着斑的turnover。在乳腺癌细胞内, 当用shRNA沉默*FLNA*表达时, 能导致迁移细胞前缘黏着斑的分解并促进细胞迁移, 并且该作用是通过ERK-MAPK信号通路激活calpain来实现的[66]。Calpain能水解包括FlnA、talin、黏着斑激酶、paxillin和Src在内的多种黏着斑蛋白, 在促进黏着斑的分解和细胞迁移中起重要作用[100-102]。但是, FlnA是如何抑制ERK活性进而影响黏着斑的？既往的研究表明, FAK(focal adhesion kinase)介导ERK对calpain的激活, 并且该通路在促进黏着斑的分解和细胞迁移方面起作用[103]。另外, SHIP2能抑制FAK活性, 并且抑制黏着斑的分解[104]。因此, FlnA可能通过SHIP2来实现其抑制钙蛋白酶、抑制黏着斑分解和促进细胞迁移的作用。

Turnover是黏着斑在片状伪足内不断分解和重新生成的过程, 整合素的内吞和胞吐在黏着斑turnover的过程中起到了重要作用。FlnA可能参与整合素细胞膜穴样凹陷介导的内吞(caveolae-mediated endocytosis)。整合素$\alpha_v\beta_3$、$\alpha_5\beta_1$和$\alpha_L\beta_2$能通过细胞膜穴样凹陷被内吞到细胞内[105-107], 并且该内吞作用影响白细胞的迁移[107]。PKCα在调控整合素$\alpha_2\beta_1$细胞膜穴样凹陷介导的内吞中起重要作用[107]。FlnA与细胞膜穴样凹陷蛋白-1(caveolin-1)相互作用, 调控细胞膜穴样凹陷介导的内吞[40,108], 并且该作用依赖PKCα对FlnA的磷酸化[40]。上述研究结果提示, FlnA可能通过caveolin-1在PKCα介导的整合素内吞的过

程中起作用。

除了可能在整合素内吞的过程中起作用外, FlnA还参与整合素的胞吐, 将细胞质内的整合素循环回细胞膜表面, 以生成新的黏着斑, 并调控细胞的迁移[20-22]。具体的过程如下: 整合素内吞后与波形蛋白寡聚体结合, 被扣留在细胞质内, 在受到细胞内信号刺激时, FlnA作为支架蛋白介导PKCε对波形蛋白的磷酸化, 磷酸化后的波形蛋白组装到中间丝上, 并将整合素释放到细胞膜表面。

7 展望

越来越多的研究表明, FlnA在细胞与基质黏附和迁移的过程中起关键作用。细胞迁移在发育、伤口愈合、炎症反应和肿瘤转移等多种病理生理过程中发挥重要作用, 由FlnA功能异常导致的细胞迁移障碍更是多种遗传性疾病以及肿瘤转移的重要发病机制。然而, FlnA在不同的细胞类型和不同的刺激下, 作用的信号通路有所不同, 影响的细胞结构也不一样, 从而对迁移产生的作用也不尽相同。FlnA作为一个支架蛋白, 可以与众多的其他分子相互作用, 因此, 在研究FlnA对迁移的影响的过程中, 我们还需要明确这些FlnA结合蛋白的作用。另外, FlnA与其他分子相互作用受磷酸化、蛋白水解等多种机制的调控, 对这些方面的进一步研究也将会加深我们的理解。

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