上皮–间充质化在恶性黑色素瘤中的研究进展

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摘要 恶性黑色素瘤是恶性程度极高的肿瘤, 一旦转移致死性极高。上皮–间充质化(epithelial-mesenchymal transition, EMT)在恶性黑色素瘤的侵袭转移、耐药、免疫抑制等过程中扮演重要角色。多种相关因子, 例如: E-钙黏蛋白、N-钙黏蛋白、Twist、Snail、Slug、Zeb1、Zeb2、波形蛋白及miRNA等参与了EMT过程。该文回顾了EMT在恶性黑色素瘤中的研究进展, 目的在于深入发掘EMT在恶性黑色素瘤中的作用, 进一步探讨治疗恶性黑色素瘤潜在的新方法。

关键词 上皮–间充质化; 恶性黑色素瘤; 肿瘤侵袭转移; 耐药; 免疫抑制

Research Progress of Epithelial-Mesenchymal Transition in Malignant Melanoma

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Abstract Malignant melanoma (MM) is the most aggressive skin cancer, which is deadliest once metastasized. Epithelial-mesenchymal transition (EMT) plays an important role in the process of invasion, metastasis, drug resistance and immunosuppression in MM. Some related factors such as E-cadherin, N-cadherin, Twist, Snail, Slug, Zeb-1, Zeb-2, vimentin and microRNAs associate with the EMT. Here we reviewed recent studies that aimed to better understand the role of EMT in the process of MM, and discussed opportunities for novel approaches for MM therapy.

Key words epithelial-mesenchymal transition; malignant melanoma; invasion and metastasis; drug resistance; immunosuppression

恶性黑色素瘤(malignant melanoma, MM)是源于黑色素细胞的恶性肿瘤。在过去的20年中, 世界范围内恶性黑色素瘤的发病率增加了2倍[1-2]。皮肤是恶性黑素色瘤的主要发病部位, 占皮肤恶性肿瘤死亡率的约75%[3]。侵袭与转移是恶性黑色素瘤患者死亡的主要原因, 而上皮–间充质化(epithelial-mesenchymal transition, EMT)在其中扮演着重要角色, 被认为是启动肿瘤侵袭转移的关键事件。大量研究表明, 经历EMT的恶性黑色素瘤细胞能从原位肿瘤逃离, 侵袭到周围组织, 经过血液或淋巴途径到达远处器官形成转移灶。值得关注的是, 新近研究发现, 拥有EMT表型的恶性黑色素瘤细胞更具耐药、

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免疫抑制及类干细胞等攻击特性。

1 上皮–间充质化

EMT是上皮表型细胞转变为间质表型细胞的过程[4]。EMT允许细胞丢失上皮顶–底极性及细胞紧密连接, 获得间质纺锤样形态和松散细胞连接, 使细胞在空间上不受细胞外基质限制, 成为具有运动性和侵袭性的间质或类间质细胞。根据环境背景的不同, EMT分为3型: I型EMT主要与胚胎形成和器官发育密切相关; II型EMT主要影响炎症、损伤修复和组织再生; III型EMT则在肿瘤发生侵袭转移中扮演重要角色[5]。

III型EMT, 又被称为致癌肿型EMT, 发生在上皮肿瘤细胞中。目前标记肿瘤细胞EMT的主要依据为: 上皮标志蛋白如E-钙黏蛋白(E-cadherin)、角蛋白(cytokeratin)、紧密连接蛋白(claudin)、闭合蛋白(occludin)等的表达下降或缺失, 间质标志蛋白如波形蛋白(vimentin)、纤连蛋白(fibronectin)、N-钙黏蛋白(N-cadherin)等表达增高; 肿瘤细胞出现成纤维细胞样形态变化; 肿瘤细胞运动性、侵袭性增强等。

在恶性黑色素瘤中, 肿瘤细胞EMT的形成机制复杂, 多种转录因子, 如: Snail/Slug家族、Twist、EF1/ZEB1、SIP1/ZEB2等可作为“分子开关”直接调节恶性黑色素瘤EMT过程[6-8]。TGF-β、NF-κB、Wnt等信通路参与介导恶性黑色素瘤EMT, 进而促进侵袭转移[9]。此外, 新近研究发现, 大量miRNA, 如: miR-200家族、miR-9、miR-137、miR-205等可靶向调节EMT相关因子及信号通路, 在恶性黑色素瘤EMT过程中发挥重要作用[10-13]。

2 EMT在恶性黑色素瘤中的作用

2.1 EMT参与侵袭转移

转移是导致恶性黑色素瘤死亡的最主要原因。大量研究证实, 经历EMT的恶性黑色素瘤细胞更具侵袭转移特性, 衰减肿瘤EMT可有效遏制恶性黑色素瘤进展[14-15]。

研究表明, 在恶性黑色素瘤细胞系中, 酸性微环境和脂肪微粒通过上调间质标志物, 抑制E-cadherin, 触发EMT程序, 进而增强恶性黑色素瘤的迁移和肺转移能力[16-17]; P53家族抑制剂高表达后, 下调E-cadherin、上调Slug, 可促进恶性黑色素瘤细胞EMT表型, 进而驱使肿瘤迁移、侵袭和转移[18]; 在恶性黑色素瘤细胞系及小鼠动物模型中, Nox1通过诱导EMT进而提高恶性黑色素瘤细胞的迁移和侵袭能力[19-20]。此外, 过表达PTX3能衰减多种间质类标志蛋白, 抑制EMT进程, 进而有效遏制恶性黑色素瘤细胞侵袭和转移潜能[21]。

近年来, 某些制剂已被证实能通过抑制肿瘤EMT对抗恶性黑色素瘤的侵袭、转移。在恶性黑色素瘤A375细胞系中, 桦木酸(betulinic acid, BA)通过调节EMT可有效遏制细胞侵袭表型[22]。用木犀草素处理过的恶性黑色素瘤小鼠模型, E-cadherin表达增加, vimentin表达降低, EMT进程受阻滞, 肿瘤肺转移灶显著减少[23]。此外, 在小鼠转移模型中, 二甲双胍能调节EMT相关转录因子Slug、Snail, 下调N-cadherin, 进而抑制恶性黑色素瘤侵袭转移[24]。

因此, EMT在恶性黑色素瘤侵袭转移过程中发挥关键作用, 遏制促进EMT发生的相关因子及通路可能成为恶性黑色素瘤治疗的重要手段。

2.2 EMT参与耐药

肿瘤耐药是常规放、化疗及免疫治疗失败的主要原因, 也是患者预后不良的指标。新近的研究提示, EMT和肿瘤耐药密切相关, 发生EMT的恶性黑色素瘤细胞耐药性增加, 而抑制肿瘤细胞EMT则可能逆转肿瘤细胞耐药。

研究表明, 在恶性黑色素瘤细胞系中过表达EMT相关基因*Snail1*, 可促进耐药发生[25]; 在小鼠B16F10恶性黑色素瘤模型中, 肿瘤相关基因(cancer associated gene, *CAGE*)参与诱导Snail的表达, 促进EMT, 进而对抗肿瘤药物雷公藤素产生耐药[26]。更为重要的是, 研究发现, 纯化的恶性黑色素瘤干细胞(melanoma stem cell, MSC)中EMT相关因子高表达, 提示EMT和恶性黑色素瘤干细胞之间存在重叠效应(overlap), 这和肿瘤耐药密切相关[27-28]。

然而, EMT介导恶性黑色素瘤耐药形成的机制, 至今远未阐明。因此, 深入探索EMT与恶性黑色素瘤耐药的分子机制, 可能为恶性黑色素瘤耐药早期阶段干预相关靶基因的新药物设计和新基因治疗方法的建立提供理论依据。

2.3 EMT参与免疫抑制

肿瘤可诱导机体产生免疫抑制细胞, 对机体抗肿瘤免疫应答起负性调节作用, 即免疫抑制作用, 这是肿瘤免疫逃逸的主要机制之一。相关研究证实, 具EMT表型的恶性黑色素瘤细胞能诱导机体产生免

疫抑制, 而抑制肿瘤细胞EMT则能增强机体免疫应答能力。

在小鼠恶性黑色素瘤模型中, 乳脂球表皮生长因子(milk fat globule EGF-8, MFG-E8)能触发恶性黑色素瘤细胞经历EMT, 出现免疫抑制效应, 最终导致肿瘤细胞凋亡受阻, 侵袭性增强[29]。Snail是主要的EMT相关转录因子, 研究证实, 鼠和人类恶性黑色素瘤细胞系导入Snail能成功诱导EMT过程。进一步结果显示, 具有EMT典型特性的Snail+黑色素瘤细胞能在体内、外诱导免疫抑制性细胞因子、调节性T细胞(Treg), 损伤树突细胞, 同时介导细胞毒性T细胞抵抗, 有助于机体获得免疫抑制, 进而促进黑色素瘤转移; 相反, 在恶性黑色素瘤瘤体内注射Snail特异性siRNA, EMT可得到有效逆转, 使肿瘤特异性浸润淋巴细胞和系统性免疫应答增加[30]。

因此, 经历EMT的恶性黑色素瘤细胞有助于机体获得免疫抑制特性, 进而促进肿瘤恶性进展。靶向调节EMT相关因子有望恢复恶性黑色素瘤病人的免疫活性, 进而提高患者生存质量, 延长患者的生存期。

3 恶性黑色素瘤EMT的相关通路

3.1 TGF-β通路

TGF-β信号通路是介导恶性黑色素瘤细胞EMT过程中最为重要的信号通路之一, 可分为Smad依赖型和Smad非依赖型。在Smad依赖型TGF-β信号通路中, TGF-β先活化Smad2和Smad3, 而后与Smad4形成复合物, 复合物进入胞核后与EMT相关蛋白转录因子结合, 有助于肿瘤细胞获得EMT表型, 促进肿瘤细胞迁移、侵袭转移[31-34]。非依赖Smad信号通路中, TGF-β通过SPARC等因子调节EMT过程。

大量研究提示, 在乳腺癌、肺癌、结肠癌、前列腺癌等细胞系中TGF-β通路均有助于肿瘤细胞形成EMT表型, 进而增强细胞的迁移、侵袭和转移能力[31,35-36]。同样, 在恶性黑色素瘤中, TGF-β/Smad的直接靶点GLI2联合ZEB1能显著抑制E-cadherin表达, 诱导EMT形成, 进一步促进恶性黑色素瘤细胞侵袭转移[37-38]。Nodal作为TGF-β家族的一员, 在B16鼠恶性黑色素瘤中, 其高表达可伴随肿瘤细胞EMT改变[20]。此外, Smad非依赖型信号通路可通过SPARC维持Slug高表达, 同时抑制E-cadherin的表达, 调节EMT, 诱导恶性黑色素瘤细胞迁移[39-40]。

因此, TGF-β信号通路激活有助于恶性黑色素EMT形成, 而抑制信号活性能有效遏制EMT进程, 从而调节恶性黑色素瘤疾病进展。

3.2 Wnt信号通路

大量研究指出, Wnt信号通路在恶性黑色素瘤细胞EMT过程中亦起重要作用, 分为典型和非典型信号通路。在典型Wnt通路中, Wnt活化使β-连环蛋白(β-catenin)从“破坏复合物”(destruction complex)中游离出来, 易位到细胞核形成β-catenin–LEF/TDF复合物, 诱导下游基因转录参与调节EMT过程[41-42]。非典型Wnt信号通路通过和相关配体结合进而参与恶性黑色素瘤EMT过程。

研究显示, 在乳腺癌、肝癌、浆液性卵巢癌中, Wnt通路均能通过介导EMT相关转录因子, 促进EMT形成, 进而促进肿瘤进展[22-23,43-44]。在恶性黑色素瘤中, 活化典型Wnt通路可诱导黑色素瘤细胞EMT进程, 并伴随肿瘤侵袭转移能力增强[45]。非典型Wnt通路也参与调节EMT过程, 例如, Wnt5a是非典型Wnt配体, 在恶性黑色素瘤中可上调vimentin、Snail表达, 降低E-cadherin水平, 促进EMT产生, 有助于恶性黑色素瘤的侵袭、转移[46-47]。

可见, Wnt信号通路是恶性黑色素瘤细胞EMT形成的重要信号通路。Wnt通路中促进EMT进程的关键靶分子有望成为阻止恶性黑色素瘤进展的重要治疗靶点。

3.3 NF-κB信号通路

NF-κB信号通路在恶性黑色素瘤细胞EMT过程中扮演关键角色。活化的NF-κB信号能在细胞核中与EMT相关Snail、ZEB等转录因子相互作用, 促进肿瘤恶性进展[48-49]。

在乳腺癌、非小细胞肺癌中, NF-κB信号通路可诱导肿瘤细胞EMT进程, 促进肿瘤细胞侵袭和转移[50-53]。同样, 在恶性黑色素瘤细胞系中, NF-κB信号活化可上调Snail蛋白, 抑制E-cadherin的表达, 诱导恶性黑色素瘤细胞EMT, 促进肿瘤进展[54]。

此外, 大量实验证实, 相关药物能通过靶向NF-κB通路抑制肿瘤细胞EMT进程, 进而抑制恶性黑色素瘤进展。例如, 在恶性黑色素瘤细胞系中, 非瑟酮可靶向NF-κB信号通路, 有效逆转EMT过程, 抑制恶性黑色素瘤细胞的侵袭性[3]; 在恶性黑色素瘤小鼠模型中, 白藜芦醇能下调NF-κB活性, 抑制肿瘤细胞EMT形成, 在一定程度上阻止癌肿恶性进展[55]。

由此可见, NF-κB信号通路在促进恶性黑色素瘤EMT过程中发挥作用, 成为恶性黑色素瘤侵袭、转移的驱动因素之一。靶向抑制NF-κB信号通路, 衰减EMT有可能成为阻止恶性黑色素瘤进展的关键所在。

4 miRNA调节恶性黑色素瘤EMT

microRNA(miRNA)是一类长度约21~23个核苷酸的调控性非编码小分子RNA, 可通过mRNA剪切和抑制蛋白质翻译等方式于转录后负调控靶基因。大量研究证实, miRNA通过调节EMT“开关分子”及相关信号通路参与恶性黑色素瘤EMT过程, 在恶性黑色素瘤侵袭转移中发挥重要作用。

研究显示, miR-205过表达可引起ZEB2降低, 伴随E-cadherin水平升高, 进而调节恶性黑色素瘤迁移、侵袭特性[12]; miR-200a、miR-203表达丢失能显著降低恶性黑色素瘤E-cadherin的表达, 从而促进恶性黑色素瘤进展[11]。此外, miR-137能靶向转录协阻抑物羧基端结合蛋白1(carboxyl-terminal binding protein 1, CtBP1)影响下游E-cadherin和Bax表达, 抑制EMT过程, 从而诱导恶性黑色素瘤细胞的凋亡[13]; miR-9过表达能抑制NF-κB1-Snail信号通路, 上调E-cadherin表达, 抑制肿瘤EMT表型, 进而显著抑制恶性黑色素瘤细胞增殖、转移[10]。值得关注的是, 在恶性黑色素瘤肿瘤疫苗研究过程中发现, 辅助过表达miR-200c, 敲除*ZEB1*, 能有效提高抗恶性黑色素瘤疗效。这一效应可能和EMT相关分子, E-cadherin、vimentin、N-cadherin表达改变有关[56]。

因此, 在治疗恶性黑色素瘤过程中, 在靶向EMT相关分子的同时联合miRNA作用, 有望进一步提高恶性黑色素瘤的治疗效果。

5 总结

综上所述, EMT是多因素多步骤的发展过程, 与恶性黑色素瘤的侵袭转移、耐药、免疫抑制等密切相关。多种转录因子、信号通路及miRNA等均参与调节EMT进程。由于恶性黑色素瘤对传统放、化疗不敏感, 且极易出现耐药, 靶向抑制促进EMT发生的相关因子、抑制恶性黑色素瘤侵袭转移、增强患者免疫活性等, 可能成为未来恶性黑色素瘤靶向治疗的新方向[57]。所以, 深入发掘EMT在恶性黑色素瘤中的作用机理, 发现抑制EMT进程的关键靶分子, 确立恶性黑色素瘤治疗的新靶点成为恶性黑色素瘤研究中亟需解决的问题。

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