Raf-1激酶与肿瘤治疗

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摘要 自Raf激酶被证明为逆转录病毒致癌基因的产物以来, 逐渐成为人们研究的热点。研究表明, Raf激酶既是Ras的效应物, 又能作为ERK信号通路中的重要组分, 成为活化的Ras和ERK之间的一个重要纽带。Ras-Raf-MEK-ERK信号通路参与了细胞增殖、分化和凋亡等生物学过程。作为这一信号通路上的节点蛋白, Raf激酶在肿瘤发生过程中起着关键作用。Raf家族成员Raf-1(c-Raf)在调控细胞运动和凋亡过程中发挥关键作用, 它既可以通过抑制促凋亡激酶ASK1和MST2活性来抑制细胞凋亡, 也可以通过激活Rok-α的活性来促进细胞迁移。该文主要综述了Raf-1激酶的调控机制及其在肿瘤发生过程中的作用, 同时也总结了以Raf-1为靶点的肿瘤治疗的最新进展。

关键词 Raf-MEK-ERK信号通路; Raf激酶; 肿瘤治疗; Raf-1; 凋亡; 细胞迁移

Raf-1 Kinase and Tumor Therapy

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Abstract Since Raf kinases were identified as products of retroviroal oncogenes, the Raf kineses have been intensively studied. Raf kinases are not only the bona fide effectors of the Ras, but also activators of the typical ERK signaling pathway, which provide an important link between Ras and ERK. Ras-Raf-MEK-ERK signaling pathway is involved in many biological process including cell proliferation, differentiation and apoptosis. As a node of the Ras-Raf-MEK-ERK signaling pathway, Raf kinases play a crucial role in tumorgensis. Raf-1, a key member of Raf family, regulates cell motility by controlling the activity of Rok-α, and apoptosis by suppressing the activity of the proapoptosis kinases, ASK1 and MST2. In this review, we described the regulation of Raf-1 kinase and its role in the tumorgensis. We also summarized the latest development in tumor therapy targeting Raf-1.

Key words Ras-Raf-MEK-ERK signaling pathway; Raf kinases; tumor therapy; Raf-1; apoptosis; cell mobility

Raf激酶家族有3个成员: Raf-1/C-Raf、B-Raf和A-Raf。三个家族成员都与肿瘤发生相关。Raf-1是首先被验证的亚型[1], 它在调控生长因子信号通路的细胞效应方面发挥关键作用[2-4]。Raf-1能介导肿瘤细胞的增殖、分化和凋亡等多种生理过程, 对肿瘤的发生和发展有重要促进作用[5]。本文主要介绍了Raf-1参与肿瘤发生的机制, 并综述了以Raf-1为靶点的肿瘤治疗的最新研究进展。

1 Raf-1的结构以及调控

1.1 Raf-1的结构

Raf激酶是由*raf*基因编码的蛋白产物, 其活化

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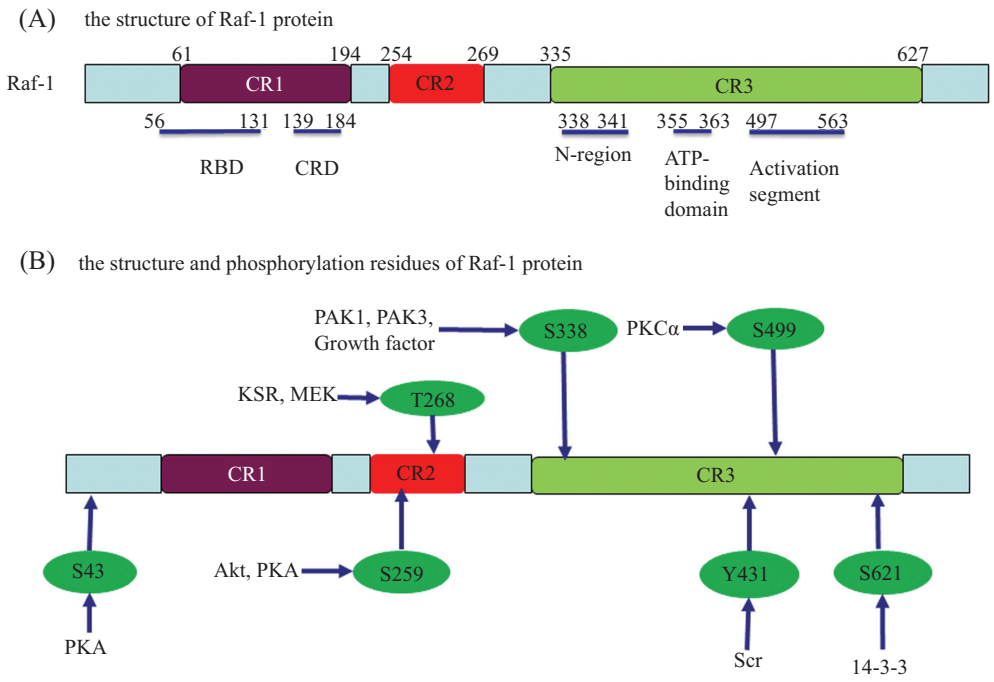

图1 Raf-1蛋白的结构以及磷酸化调控位点

Fig.1 Structure and phosphorylation sites of Raf-1 protein

后具有丝/苏氨酸蛋白激酶活性。其中Raf-1由648个氨基酸组成, 分子量约为74 kDa, 是目前为止研究最广泛、最深入的亚型。Raf激酶家族结构类似, 其分子结构都包含3个保守区域(conservative region, CR)(图1)。CR1包含Ras结合域(Ras-binding domain, RBD)和半胱氨酸富集域(cysteine-rich domain, CRD)。CR2含有重要的磷酸化位点, 它参与了Ras结合以及Raf激活的负调控[6]。CR3为激酶结构域, 包含激活区域, 对于激酶激活起着关键作用[7]。随着近年来分子生物学的发展, RBD的结构域、Raf-1的CR1拓展结构域[8-10]以及B-Raf[11]和Raf-1[12]的CR3结构域的功能已经被成功鉴定。这为以Raf为靶点的肿瘤治疗奠定了理论基础。

1.2 Raf-1的调控

Raf-1的调控是以激活/去激活循环的方式进行的。在Raf-1处于非激活状态时, Raf-1以封闭环的形式存在。封闭环是N端调控区域折叠封闭了催化区域而形成[13]。当激素、生长因子等与细胞表面受体结合时, 细胞中的Ras-GTP增加, 进而激活Ras; Ras的激活会引发Ras-GTP与Raf-1直接结合, 从而将细胞浆中的Raf-1二聚体募集到细胞膜上。募集到膜上的Raf-1发生磷酸化而激活。最终, 激活的Raf-1与蛋白磷酸化酶PP5特异性结合从而使得Raf-1发生去磷酸化, 导致Raf-1回到非激活状态。

2 Raf-1与肿瘤发生

2.1 Raf-1对细胞凋亡的调节

2.1.1 对线粒体途径的调控 Raf-1可以通过线粒体途径来发挥抑制细胞凋亡的作用[14-16]。当凋亡抑制蛋白Bcl-2过表达时, Raf-1被Bcl-2激活并将抑制细胞凋亡信号传递至线粒体[17]。此外, 部分生长因子和p21活化激酶(P21-activated kinases, PAK)共同参与了Raf-1激酶的S338位点的磷酸化[18-19], 进而促进Raf-1转位至线粒体。Raf-1介导了Bcl-2家族成员促凋亡蛋白BAD的磷酸化失活[17]。此外, Raf-1作为支架蛋白来募集蛋白激酶C-θ(PKC-θ), 进而诱导BAD磷酸化[20]。Raf-1与线粒体的电压依赖性阴离子通道(voltage dependent anion channels, VDACs)直接相互作用, 可能抑制了细胞色素C从线粒体向胞浆的释放[21]。且与Raf激酶抑制蛋白RKIP分离的Raf-1转位至线粒体, 抑制了细胞凋亡, 最终促进癌症发生[22-23](图2)。

2.1.2 对Fas激酶的调控 Fas是肿瘤坏死因子受体家族成员, 广泛存在于组织细胞。研究显示, $Rock_2$激酶活性增高可促进细胞骨架连接蛋白(ezrin)发生磷酸化而激活, 进而促进Fas在Raf-1敲除的胚胎成纤维细胞的细胞膜上募集和表达, 最终促进细胞凋亡[24-25]。Fas活化可促进Raf-1-$Rock_2$络合物的形成, 下调$Rock_2$激酶的活性。因此, Raf-1通过与$Rock_2$直

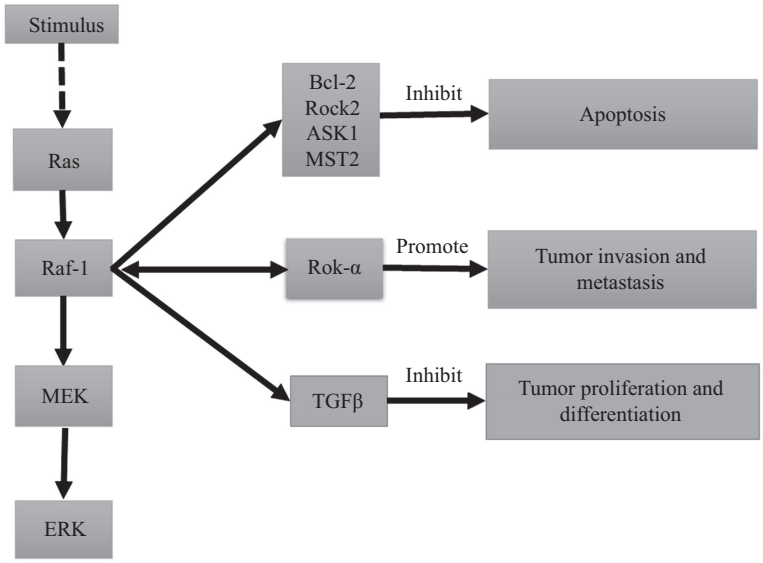

图2 与肿瘤发生相关的Raf-1信号途径

Fig.2 Tumor-related signalling pathways of Raf-1

接结合来降低$Rock_2$的活性, 从而反馈性地抑制Fas在细胞膜表面的表达, 最终抑制细胞凋亡的发生(图2)。

2.1.3 对ASK1激酶和MST2激酶的调控 Raf-1在抑制细胞凋亡过程中其他的作用靶点是促凋亡激酶: 细胞凋亡信号调节激酶1(apoptosis signal-regulating kinase 1, ASK1)和磷酸化蛋白激酶2(mammalian Sterile20-like kinase 2, MST2), Raf-1直接与之结合, 并抑制该激酶的活性。这些抑制效应不需要Raf-1激酶的活性而是通过Raf-1与促凋亡激酶直接结合而介导的。ASK1位于JNK MPAK和p38 MPAK上游, 通过激活死亡受体(如TNF-α、Fas)途径来促进细胞凋亡[26-27]。在人内皮细胞中, Raf-1通过结合并抑制线粒体中ASK1的激酶活性, 抑制细胞凋亡的发生。在大鼠心脏中特异性的敲除*raf-1*基因, 导致由心肌细胞凋亡增加而引起的心室扩大症和纤维症, 同时, 敲除*ASK1*能抑制上述症状的发生。以上研究阐明了Raf-1对ASK1的抑制效应的病理生理学意义[28]。

Raf-1与MST2的SARAH结构域结合, 进而干扰了MST2二聚化作用及募集。Raf-1激酶失活的突变体也可以抑制MST2激活。MST2活性在*raf-1*敲除的细胞中显著提高[29]。这些都表明了Raf-1可以直接与ASK1激酶和MST2激酶结合, 进而抑制它们的活性, 最终发挥抑制细胞凋亡的作用(图2)。

2.2 Raf-1促进肿瘤细胞的运动

Raf-1可通过下调Rho激酶-α(Rok-α)的活性来促进肿瘤细胞的运动[25]。*raf-1*敲除的细胞中, 使用Rok-α的化学抑制剂抑制Rok-α的活性或诱导Rok-α的负调控结构域发生突变, 可以逆转因*raf-1*敲除而对细胞运动的抑制作用, 该结果表明, 在细胞运动过程中Rok-α是Raf-1的唯一作用靶点[25]。对其机理的研究发现, Raf-1半胱氨酸富集域(CRD)在该过程中起到了至关重要的作用。Rok-α像Raf-1一样, 具有自身抑制活性的特性, 其C端调控区域高度类似于Raf-1的CRD。Raf-1的CRD调控结构域能够与Rok-α激酶结构交联结合, 并抑制Rok-α激酶的活性, 进而促进肿瘤细胞的运动[30]。Raf-1和Rok-α相互作用的生物学相关性在Ras诱导的小鼠皮肤肿瘤模型中得了很好的验证[31](图2)。

2.3 Raf-1抑制肿瘤细胞的增殖与分化

Raf-1可以通过诱导转化生长因子-β(transforming growth factor-β, TGF-β)产生来抑制肿瘤细胞的增殖与分化。TGF-β是一个抑制肿瘤细胞增殖的关键诱导物。许多肿瘤通过下游调控或TGF-β受体突变体来使TGF-β信号转导失效, 或通过使其下游靶点失活, 如视网膜母细胞瘤蛋白Rb, 从而使其迅速增殖[33]。而激活的Raf-1则可以通过诱导TGF-β的产生来抑制肿瘤细胞的分化和凋亡[34-35]。

此外, Raf-1可以通过Raf/MEK/ERK级联激酶通路参与细胞分化的调节。ERK1/2影响细胞分化可能与M期细胞内微管结构有关[36]。最近研究表明, 在表皮肿瘤中, 内源性Raf-1对于维持表皮肿瘤细胞未分化状态至关重要。激活的的Rho激酶-α(Rok-α)

可以诱导Raf-1有条件消融, 进而导致已有肿瘤通过MEK/ERK依赖性激活一个分化程序[37](图2)。

3 以Raf-1为靶点的肿瘤治疗的相关研究

3.1 癌症中的*raf-1*基因突变

Raf作为Ras的关键下游效应因子参与肿瘤的发生和发展。Raf激酶家族成员Raf-1在肿瘤的发生与发展中的作用一直是人们研究的主要目标。与癌症相关的Raf-1突变体, 如S247G突变体和E478K突变体, 也已经被广泛报道。它们先后在化学药剂诱导的大鼠肺癌模型、人肿瘤细胞株以及人急性髓细胞样白血病(therapy-related acute myeloid leukemia, t-AML)中被报道[37]。在人t-AML病人中, *raf-1*基因突变率很高, 但*raf-1*突变只表现出轻微的促进癌细胞转化能力。通过对t-AML病人的ERK通路的激活情况分析发现, 只在恶性肿瘤组织中检测到*raf-1*突变, 而在周围正常组织中没有检测到*raf-1*突变。最近报道表明, 在人类黑色素瘤中发现了Raf-1突变体E478k。在体外结肠癌模型中, Raf-1 E478突变体对*ras*癌基因激活高度敏感。这个突变体功能与B-Raf功能相似, 在胃癌、肺癌和乳腺癌细胞中也发现了Raf-1 jjjjE478k突变体[38]。除了*raf-1*基因的突变, Raf-1家族成员的其他变异也在人类恶性肿瘤中被报道。*B-Raf*和*raf-1*基因重排和融合已经在在甲状腺瘤、纤维性星形细胞瘤、前列腺癌、子宫内膜瘤、黑色素瘤中被报道[38-42]。

3.2 以Raf-1为靶点的癌症治疗

抑制Ras信号以治疗癌症的临床前及临床研究失败后, 以Raf蛋白家族及其下游效应器为靶点的药物研发逐渐成为人们研究的重点。药物研发采用如下策略: 采用小分子抑制剂来抑制Raf激酶的活性; 或者利用反义寡核苷酸来减少Raf蛋白的表达水平; 或者以Raf蛋白与其他蛋白之间的相互作用为目标, 尤其是Raf-Ras之间相互作用。第一个研发出来的药物就是Raf-1的抑制剂, 随着B-Raf激活的突变体在肿瘤中越来越多地被发现, 人们也开始致力于研发B-Raf和MEK1/2的抑制剂。

3.2.1 Raf激酶抑剂(表1) 索拉非尼(BAY 43-9006)是第一个通过临床试验的Raf抑制剂。临床前试验显示, 在肿瘤细胞株以及Ras激酶依赖型肿瘤的异种移植模型中, 索拉非尼能显著抑制Raf-1和B-Raf的活性[43-44]。进一步研究表明, 索拉非尼可以用于治疗晚期肾细胞癌(renal cell carcinoma, RCC)以及原发性肝癌(hepatocellular carcinoma, HCC)。然而, 索拉非尼单体或联合用药对于黑色素瘤尤其是携带B-Raf突变的恶性黑色素瘤的临床治疗效果不是很理想[45-46]。索拉非尼作为一种特异性的Raf-1激酶抑制剂被研发, 尽管其对突变的B-Raf不能充分抑制, 但它对血管内皮生长因子(vascularendothelial growth factor, VEGF)和血小板衍生生长因子(platelet derived growth factor, PDGF)受体激酶具有高度抑制效应[47]。索拉菲尼作为二线抗肿瘤药物, 正在进行大规模多中心临床试验, 尽管临床试验取得了较好的临床效果, 然而如何发挥该药物的最大疗效尚不明确。

AZ628是野生型Raf-1和V600E B-Raf的选择性抑制剂。在许多细胞中, 尤其是在含有V600E B-Raf

表1 Raf-1抑制剂

Table 1 Inhibitors of Raf-1

名称 Compound	肿瘤 Tumors
Sorafenib	Leukemia, Hepatocellular carcinoma (HCC), Renal tumor, Pancreatic cancer, Bladder cancer, Lung cancer, Neuroendocrine tumors, Thyroid carcinoma, Squamous cell carcinoma
LErafAON (NeoPharm, Inc)	Advanced malignant tumor
ISIS 5132 (Isis Pharmaceuticals)	Ovarian cancer, Breast cancer
Dabrafenib	Gastrointestinal stromal tumors, Papillary thyroid carcinoma, Non-small cell lung cancer, Ovarian cancer, Colorectal carcinoma
AZ628	Melanoma
GW5074	Huntingdon's disease
ZM336372	Pheochromocytoma, Medullary thyroid carcinoma Hepatocellular carcinoma
PLX-4720	Colon cancer, Renal cell carcinoma, Hepatocellular carcinoma, Melanoma
NVP-BHG712	Melanoma, Malignant glioma
CEP-32490	Colon cancer, Medullary thyroid carcinoma

突变的细胞中, 该抑制剂发挥明显的细胞毒作用。

我们的研究表明, 以Raf与其他蛋白相互作用为靶点的药物研发有着光明的前景[48]。MCP-110作为抑制Raf与Ras相互作用的小分子化合物, 能显著抑制表达Ras的悬浮细胞的生长, 但不抑制表达Raf-1的细胞的生长, 表明MCP-110特异性抑制Ras-Raf之间的相互作用[49]。Raf与肿瘤抑制蛋白Rb的相互作用是药物作用的另一个靶点。研究表明, Raf-1结合并诱导Rb磷酸化, 进而介导Rb的抑制以及S期发展[50]。在细胞株中小分子的合成肽阻断了Raf-1-Rb相互作用, 从而抑制A549异种移植肿瘤的生长[51]。合成肽作为药物的应用, 因其短暂的半衰期以及其运输传递问题而受到限制, 小分子药物RRD-251具有相同的疗效。该药物依赖完整的Rb表达水平, 它在体外能抑制肿瘤细胞增殖、异种移植瘤的形成和肿瘤血管生成[52]。尽管没有临床数据支持, 但这些实验结果依然表明, 以Raf-1-R相互作用为靶点可能是一个成功的抗癌策略。

3.2.2 反义寡核苷酸 反义核苷酸是人工合成、化学修饰的短片段的DNA或RNA, 主要通过Watson-Crick碱基配对与编码目的蛋白的mRNA相互作用, 从而抑制蛋白表达。利用反义寡核苷酸来抑制Raf的表达是一个进步。ISIS 5132是一个含有20个核苷酸的反义寡核苷酸, 能抑制*Raf-1*的mRNA的表达。它在前期临床试验中抑制肿瘤的发育, 而二期临床实验表明, 其单独使用时没有任何作用[49]。LErafAON是由15个核苷酸组成的反义寡核苷酸的脂质体衍生物[49]。用脂质体包裹反义寡核苷酸的方法可以保护寡核苷酸远离核苷酸酶, 并有利于在细胞中的运输。然而, 临床一期试验证明, 脂质体的存在影响了药物的靶向性, 且增加了药物的副作用[53-54], 因此, 利用该方法抑制Raf的观点遭到质疑。随着运输传递方式的研究发展, 脂质体包裹反义寡核苷酸技术可能会有所突破。

3.2.3 Raf抑制剂的反常效应 RAF激酶家族能调节细胞生长、分化以及生存等一系列生命过程。当用Raf抑制剂ZM 336372处理细胞时, 体外分离和检测Raf激酶发现ZM 336372介导了一系列反常的Raf激酶激活过程[55]。Therrien及其同事Sicheri等[56]首次证实, 两个Raf蛋白发生二聚化对激活该蛋白至关重要。如果抑制Raf二聚化则会阻断激活过程, 从而终止癌细胞的生长。另外的3篇文章也证明了这个观点[57-59]。我们的研究表明, Raf-1同源二聚化或Raf-1与B-Raf异源二聚化结合对激活Raf激酶至关重要[60]。当使用B-Raf特异性抑制剂, 或Ras突变介导的Raf二聚化发生时, Ras/Raf/MEK/ERK信号通路上的激酶活性都显著提高。

在高表达B-Raf突变体的细胞系中Raf抑制剂具有高效性, 并通过抑制ERK信号通路进而抑制肿瘤发生的信号转导途径[59]。体外实验中也证实, 当用B-Raf抑制剂处理肿瘤细胞时, 肿瘤细胞的增殖明显被抑制。然而, 在敲除活化的B-Raf突变体并且表达Ras突变体的细胞系中, Raf抑制剂的作用效果正好相反[57,59]。我们的研究表明, 由Raf抑制剂介导的Raf异源二聚化能够增强Raf激酶活性以及活化下游信号转导通路。这些结果表明, Raf-1和B-Raf形成一个二聚物, 只有这个二聚物的活性完全被抑制, 其下游信号通路才能被有效地抑制[60]。与此一致的是, B-Raf特异性抑制剂诱导的ERK活化不依赖Raf-1的参与[57-58]。并且B-Raf抑制剂能够激活Raf-1, 该过程不依赖B-Raf的参与[49]。在B-Raf缺失的成纤维细胞中, B-Raf特异性抑制剂能介导ERK磷酸化。由此, 我们得到这样的结论: 抑制剂和药物联合诱导的Raf-1同源二聚化能够有效增强Raf激酶活性和活化ERK信号转导通路。Heidorn等[59]在K-Ras突变并且B-Raf失活的小鼠黑色素瘤模型中得到与上述一致的结果。

4 总结与展望

随着分子生物学技术的发展, 以Raf-1为靶点的肿瘤治疗逐渐受到人们的关注和认可。由于Raf-1激酶在信号通路的调节以及许多复杂的病理生理现象调控方面发挥重要作用, 其在肿瘤治疗方面亦发挥着重要作用, 以Raf-1为靶点的治疗研究已经在临床前期和不同临床试验中应用, 并且与传统的化疗方法相比具有毒性小且特异性强的特点, 因此以Raf-1为靶点的治疗有相当大的应用前景。研究表明, 在大部分真核细胞中存在有Ras/Raf/MEK/ERK信号通路, 随着人们对Ras/Raf/MEK/ERK信号转导通路的研究, 以Raf-1为靶点的肿瘤治疗前景将逐渐扩展, 从临床研究进入临床治疗, 为以后的肿瘤治疗提供更多的手段与方法。

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