靶向TGF-β及受体的小分子抑制剂研究进展

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摘要: 转化生长因子-β (transforming growth factor-β, TGF-β) 是一组生物学活性广泛的细胞因子, 与受体结合后通过Smad与非Smad信号通路介导一系列的生物学反应, 包括促细胞上皮-间质转化、促组织纤维化、促血管生成、促肿瘤的免疫逃逸、抑癌和促癌双重作用等。由于过度活化的TGF-β及其受体所介导的信号通路在多种疾病如恶性肿瘤及组织纤维化的发生、发展中具有重要的病理生理学作用, 一些小分子抑制剂已被开发用于阻断该信号通路, 从而为控制这些疾病提供一种新颖的治疗方案。其中, TGF-β配体抑制剂吡非尼酮已成功上市用于特发性肺纤维化治疗, 治疗肿瘤、骨髓增生异常综合征的TGF-β I型受体激酶 (ALK5) 抑制剂LY2157299、EW-7197、LY3200882处于临床Ⅰ期至Ⅲ期试验阶段, 多个其他TGF-β受体相关抑制剂如SB-431542、LY2109761、TP-0427736、IN-1130等处于临床前研究阶段。本文对以TGF-β及其受体为靶标的小分子抑制剂的最新研究进展进行了综述。

关键词: TGF-β; TGF-β受体; Smad信号通路; 小分子抑制剂
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Research progresses of small molecule inhibitors targeting TGF-β and its receptors

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Abstract: Transforming growth factor-β (TGF-β) belongs to a group of biologically active cytokines that bind to its receptors to activate Smad signaling and non-Smad signaling pathways. The biological functions of TGF-β include promoting cell epithelial-mesenchymal transition, tissue fibrosis, angiogenesis and tumor immune evasion, as well as dual effects of cancer suppression and cancer promotion. Given the fact that the ligand- and receptors-mediated abnormal activation of TGF-β signaling pathways play an important role in the pathogenesis of multiple diseases such as malignant tumors and tissue fibrosis, more than a dozen small-molecule inhibitors have been developed to block the TGF-β signaling pathways, providing a novel method for controlling the development of these diseases. At present, pirfenidone, an inhibitor for TGF-β production, has been approved for treatment of idiopathic pulmonary fibrosis, while the inhibitors of TGFβRI/ALK5 for therapeutics of tumors or myelodysplastic syndromes, including LY2157299, EW-7197 and LY3200882, are in the phase I to III clinical trials, with additional ones inhibiting TGFβRs such as SB-431542, LY2109761, TP-0427736, and IN-1130 being in the preclinical phase. This paper reviews recent advances in research of small-molecule inhibitors targeting TGF-β and its receptors.

Key words: TGF-β; TGF-β receptor; Smad signaling pathway; small-molecule inhibitor

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转化生长因子-β (transforming growth factor-β, TGF-β) 最早于1978年由De Larco与Todaro[1,2]从鼠肉瘤病毒转化的小鼠成纤维细胞3T3培养液中发现, 因具有刺激正常细胞表型转化活性而命名。迄今在哺乳动物体内已发现有4种不同亚型的TGF-β (TGF-β1～4), 其中TGF-β1活性最强[3]。TGF-β受体有3型, 其中, I、II型受体属跨膜型丝氨酸/苏氨酸激酶受体, 直接参与细胞内信号转导, III型受体不含激酶活性区, 主要起调节TGF-β与II型受体结合的作用[4]。TGF-β活化的细胞内信号由Smad和非Smad相关蛋白介导。在TGF-β/Smad信号通路 (经典通路) 中, TGF-β与III型受体 (TGFβR III) 结合, 后者将TGF-β递呈给II型受体 (TGFβR II); 随后, TGF-β与II型受体二聚体结合, 进而募集I型受体 (TGFβR I, 又称ALK5) 二聚体形成异源四聚体受体复合物; 此时受体构象发生改变, II型受体自身磷酸化, 进而磷酸化并激活I型受体; 活化的I型受体磷酸化Smad2/Smad3蛋白, 磷酸化的Smad2/Smad3与Smad4结合形成异源复合物并转运至细胞核内[5,6]。TGF-β介导的非Smad信号通路 (非经典通路) 则通过上述受体激活丝裂原活化蛋白激酶 (MAPK)、磷脂酰肌醇3激酶和蛋白激酶B (PI3K/Akt)、Rho-Rock等通路传导信号入核。其中MAPK通路下游主要包括细胞外信号调节蛋白激酶1/2 (ERK1/2)、c-jun氨基末端激酶 (JNK)、p38 MAPK三条信号通路[7]。入核的Smad和/或非Smad信号调节蛋白继而与相应转录因子或辅助蛋白结合, 调控下游靶基因的转录和表达, 从而诱导一系列与多种疾病相关的生物学反应。如通过下调上皮型或血管内皮型钙黏附蛋白 (E-cadherin/VE-cadherin) 等上皮或内皮标志物表达, 上调纤维连接蛋白 (fibronectin)、α平滑肌肌动蛋白 (α-SMA)、波形蛋白 (vimentin) 等间质标志物表达, 使细胞极性消失、运动能力增强, 诱导上皮细胞的上皮-间质转化 (epithelial-mesenchymal transition, EMT) 或血管内皮细胞的内皮-间质转化 (endothelial-mesenchymal transition, EnMT) 发生, 从而促进肿瘤侵袭/转移和毛细血管增生。再如, 通过上调fibronectin、α-SMA、胶原蛋白表达, 促成纤维细胞-肌成纤维细胞转分化和细胞外基质 (ECM) 产生, 促进胶原纤维重塑、机械应力增加, 从而导致组织纤维化。此外, TGF-β还可通过促肿瘤细胞免疫逃逸等加速肿瘤的侵袭和转移[8-10]。鉴于过度活化的TGF-β及其受体所介导的细胞信号通路在肝癌、胰腺癌、骨髓增生异常综合征 (myelodysplastic syndromes, MDS) 等恶性肿瘤及组织纤维化疾病的发生、发展中发挥关键作用, 一些靶向抑制TGF-β及其受体的小分子抑制剂日益受到关注。其中, 以ALK5为靶的小分子抑制剂品种最为丰富, 这应与ALK5具有高度保守富含甘氨酸-丝氨酸序列的GS结构域 (TGFβR II为自磷酸激酶, 无GS结构域) 和独特的氢键相互作用, 可靶性好, 利于小分子抑制剂的设计、筛选有关[11,12]。本文结合该领域的关注热点及最新动态, 对TGF-β及其受体小分子抑制剂药物的研究进展作一综述, 为医药工作者提供参考。

1 靶向TGF-β的小分子抑制剂

迄今, 明确针对TGF-β配体的小分子抑制剂极少, 吡非尼酮 (pirfenidone, PFD) 是唯一代表品种。PFD的化学名为5-甲基-1-苯基-2-(1*H*)-吡啶酮(CAS号: 53179-13-8), 相对分子量 (*M*r) 185.22, 商品名艾思瑞 (Esbriet), 化学结构见图1。PFD最初由美国Marnac公司开发, 2008年起先后在日本、欧洲、印度上市, 2014年在美国经FDA批准上市, 用于特发性肺纤维化 (idiopathic pulmonary fibrosis, IPF) 的治疗[13]。PFD可抑制多种细胞中TGF-β的产生, 进而抑制成纤维细胞增殖、减少胶原蛋白合成、延缓IPF病情发展[14]。

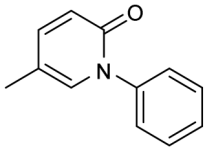


Figure 1 Structure of pirfenidone

体外研究结果显示, PFD可抑制貂肺上皮细胞CCL-64中TGF-β前体蛋白转化酶——弗林蛋白酶 (furin) 的表达, 进而降低TGF-β2 mRNA水平和成熟TGF-β2蛋白的表达[15]。在多种细胞水平模型中, PFD可抑制成纤维细胞的增殖和生物学活性, 对TGF-β诱导的胶原蛋白生成有抑制作用[16,17]。同时, PFD还能减少人外周血单核细胞产生炎症因子, 如肿瘤坏死因子α (TNF-α) 和白细胞介素1β (IL-1β) 等[18]。

体内研究结果显示, 在博莱霉素诱导的仓鼠肺纤维化模型中, PFD能降低肺羟脯氨酸表达水平及脯氨酰羟化酶活性, 对肺I、III型胶原蛋白表达均有抑制作用[19]。大量其他实验动物研究也证明PFD能缓解博莱霉素诱导的肺纤维化症状[20,21]。在低盐饮食的大鼠模型中, PFD (250 mg·kg^{-1}·d^{-1}, *p.o.*, 治疗28天) 可使慢性环孢素诱导的肾纤维化指标改善约50% (P<0.05), 并使TGF-β1蛋白表达量下降80% (P<0.001)[22]。另通过多种其他组织纤维化实验动物模型 (肺、肝、心脏、肾脏), 证明了PFD具有全身多脏器的抗纤维化活性[23]。

临床试验及上市后治疗观察显示, 对于IPF患者,

与安慰剂组比较, PFD治疗组患者的用力肺活量(FVC)绝对值下降≥10%或病死比例相对降低47.9%, FVC不降低的患者比例相对升高132.5% (P<0.001)。PFD还可显著缓解6分钟步行距离的减少 (P = 0.04)和延长无进展生存期 (P<0.001)。与安慰剂组相比, PFD治疗组在1年内全因死亡的相对风险降低48% (P = 0.01), 1年内IPF相关死亡的相对风险降低68% (P = 0.006)。在超过120周的观察期内, 与安慰剂组相比, PFD治疗组可显著降低IPF患者相关死亡率 (P = 0.023 7)[24-26]。

2 靶向TGF-β受体的小分子抑制剂

目前, 以TGF-β受体为靶的小分子抑制剂中, ALK5激酶抑制剂研究积累较为深厚, 研制机构众多, 品种丰富, 药用开发方面主要针对肿瘤、MDS、组织纤维化等, 少数品种已进入临床研究阶段。此外, 随着人们对TGF-β受体 (TGFβRs)功能认识的加深, 尤其受体蛋白晶体结构的解析, 使部分研究者开始针对配体−受体或受体−受体蛋白之间相互作用设计、筛选新型小分子抑制剂, 从而为寻找针对TGF-β信号通路的小分子抑制剂提供一些新的思路和选择。

2.1 临床研究阶段的ALK5激酶抑制剂

现处于临床研究阶段的ALK5激酶抑制剂有3个, 分别是LY2157299、EW-7197和LY3200882 (表1)。

2.1.1 LY2157299 LY2157299 (galunisertib)是美国礼来公司 (Eli Lilly and Company)研发的一个ALK5激酶抑制剂, 体外激酶抑制IC_{50}为56 nmol·L^{-1}, 最早于2005年一次国际会议中披露其研究动态[27]。LY2157299作为治疗MDS、脑/肝/胰腺癌、实体瘤的药物开发分别处于III期、II期和I期临床研究阶段, 是目前唯一一个处于临床III期试验阶段 (NCT02008318)的TGF-β受体小分子抑制剂[28]。

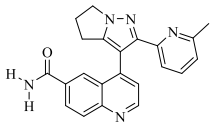
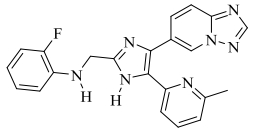
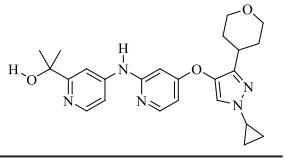
体外研究中, 对受试的人肝癌HLE细胞和HLF细胞, LY2157299可特异性下调TGF-β1诱导的Smad2蛋白磷酸化, 显著抑制癌细胞的增殖和转移。对于初级造血干细胞, LY2157299可剂量依赖性地抑制TGF-β介导的Smad2蛋白活化和造血抑制, 促进原发性MDS患者的骨髓造血功能[29]。体内研究中, 针对TGF-β过度表达的转基因骨髓造血功能衰竭小鼠模型, LY2157299 (100 mg·kg^{-1}·d^{-1}, *p.o.*, 治疗14天)可改善贫血[30]。在人非小细胞肺癌细胞Calu6和乳腺癌细胞MX1异种移植皮下荷瘤裸鼠模型中, LY2157299 (150 mg·kg^{-1}·d^{-1}, *p.o.*, 治疗20天)显示出显著的抗肿瘤活性[31]。

LY2157299治疗MDS的II期临床试验结果显示, 疗程内 (150 mg, b.i.d., *p.o.*, 治疗14天, 停药14天, 28天一个治疗周期)患者耐受性良好, 单疗程后26%的受试患者达到血液学改善, 输血需求至少降低4个单位或血红蛋白增加至少1.5 g·dL^{-1}, 并维持8周。针对晚期肝癌、神经胶质瘤患者的I期、II期临床试验结果显示, LY2157299具有良好的药代动力学性质和抗肿瘤疗效, 300 mg·d^{-1}间歇口服给药为安全剂量, 未监测到心血管毒性[32-35]。虽现有观点认为, 长期抑制TGF-β信号转导可能诱发继发性恶性肿瘤, 但LY2157299的临床评价显示, 即使接受治疗超过3年的患者也未观察到肿瘤发生的迹象[36,37]。

2.1.2 EW-7197 EW-7197 (vactosertib)是韩国MedPacto公司研发的ALK5激酶抑制剂, 最早于2012年的一篇会议摘要中报道, 目前正在进行针对MDS的II期临床试验 (NCT03074006)和晚期实体瘤的I期临床试验 (NCT02160106)[38,39]。

体外激酶实验中, EW-7197竞争性结合于ALK5胞内激酶结构域的ATP结合位点产生激酶抑制活性, IC_{50}

Table 1 Small-molecule inhibitors of activin receptor-like kinase 5 (ALK5) in clinical study

Drug	Target	CAS No.	Originator	Global status	Disease	Structure
LY2157299	ALK5	700874-72-2	Eli Lilly and Company	Phase III	Myelodysplastic syndrome	
				Phase II	Brain, liver and pancreatic cancer	
				Phase I	Solid tumor	
EW-7197	ALK5	1352608-82-2	MedPacto	Phase II	Myelodysplastic syndrome	
				Phase I	Advanced solid tumor	
LY3200882	ALK5	1898283-02-7	Eli Lilly and Company	Phase I	Solid tumor	

为11 nmol·L^{-1}。同时, EW-7197还具有ALK4抑制活性, IC_{50}为13 nmol·L^{-1}。在细胞水平上, EW-7197对小鼠乳腺上皮细胞4T1和NMuMG、人乳腺癌上皮细胞MDA-MB-231和MCF10A可剂量依赖性 (10～30 nmol·L^{-1}) 地阻断TGF-β1诱导的Smad2/Smad3蛋白磷酸化及入核, 进而抑制肿瘤细胞的侵袭和迁移[40]。在4T1、NMuMG、MDA-MB-231肿瘤细胞原位移植小鼠等实验动物模型中, EW-7197 (5～40 mg·kg^{-1}) 可抑制TGF-β1诱导的EMT过程。在小鼠乳腺瘤病毒MMTV/c-Neu转基因小鼠模型中, EW-7197 (40 mg·kg^{-1}) 可显著抑制乳腺癌的肺转移, 与模型组相比, EW-7197可减少60%的肺转移 (P<0.01)。在4T1肿瘤细胞原位移植小鼠模型中, EW-7197可增强细胞毒性T淋巴细胞 (cytotoxic T-lymphocyte, CTL) 的活化[41]。在胆管结扎大鼠模型中, EW-7197可通过阻断TGF-β/Smad信号抑制肝纤维化[42]。在小鼠B16黑色素瘤模型中, EW-7197可有效抑制黑色素瘤生长及淋巴结转移。此外, EW-7197除阻断Smad2/Smad3蛋白磷酸化, 还可通过泛素介导的Smad4蛋白降解来增强黑色素瘤小鼠$CD8^+$ T淋巴细胞增生, 进而增强抗黑色素瘤CTL免疫应答[43]。Ⅰ期临床试验结果显示, 对实体瘤患者, EW-7197 (60 mg·d^{-1}, *p.o.*, 治疗28天) 尚未发现任何严重的药物相关毒副作用[44,45]。

2.1.3 LY3200882 LY3200882是美国礼来公司研发的另一个高选择性小分子ALK5抑制剂, 可竞争性结合于ALK5激酶结构域的ATP结合位点, 首次报道于2016年ClinicalTrials.gov官网, 目前正在进行针对实体瘤的Ⅰ期临床试验 (NCT02937272)[46]。

在体外研究中, LY3200882对受试的肿瘤和免疫细胞均显著抑制TGF-β诱导的Smad蛋白磷酸化。在体外免疫抑制实验中, LY3200882可逆转TGF-β1或调节性T细胞对初始T细胞的抑制活性, 并可恢复后者的增殖能力。体内研究中, 针对三阴乳腺癌4T1-LP原位移植等小鼠模型, LY3200882表现出较强的抗肿瘤生长及转移的活性, 且活性与肿瘤微环境中浸润淋巴细胞的增强有关。此外, 在同系CT26小鼠结肠癌移植瘤模型中, LY3200882与免疫检查点抑制剂PD-L1抗体联合使用, 显示出较好的抗肿瘤活性[47]。

2.2 临床前研究阶段的ALK5激酶抑制剂

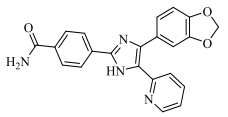
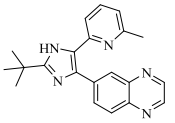
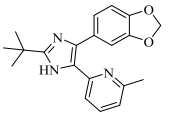
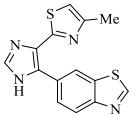
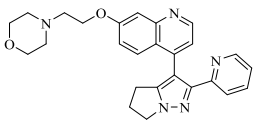
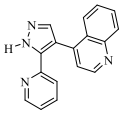
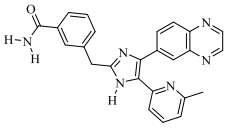
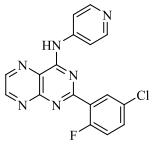
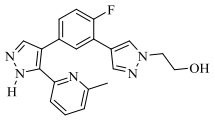
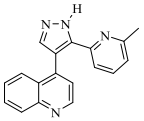
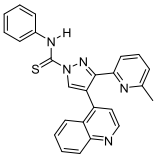
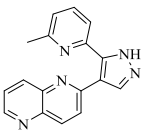
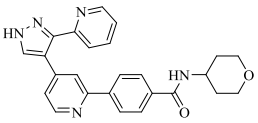
目前处于临床前研究阶段的ALK5激酶抑制剂品种较多, 包括SB-431542、LY2109761、TP-0427736、IN-1130等十几种小分子抑制剂 (表2)。从已发表的研究数据来看, 研发机构重点关注这些小分子化合物在抑制肿瘤侵袭/转移、控制瘤体毛细血管增生、增强抗瘤免疫应答、控制器官纤维化等方面的药用潜力, 对部分品种与现有抗癌化疗药的体内、外联用活性也较为关注。

2.2.1 SB-431542 SB-431542是葛兰素史克公司 (GlaxoSmithKline) 开发的一种强效ALK5抑制剂, 最早报道于2002年。SB-431542体外可抑制ALK5 (IC_{50} = 94 nmol·L^{-1}) 及ALK4、ALK7的激酶活性, 但对ERK、JNK、p38 MAPK信号通路无影响[48,49]。细胞水平研究显示, 对小鼠成纤维细胞NIH-3T3、胚胎肌母细胞C2C12、乳腺上皮细胞NMuMG以及人永生化表皮细胞HaCaT、肾上皮癌细胞A498、胰腺癌细胞PANC-1等, SB-431542 (≤10 μmol·L^{-1}) 可抑制Smad2/Smad3蛋白磷酸化水平、EMT相关生物标志物如fibronectin、α-SMA、Ⅰ型胶原蛋白、Ⅰ型纤溶酶原激活物抑制因子 (PAI-1) 的转录或表达, 并降低细胞增殖、运动能力[50,51]。体内研究显示, 在4T1肿瘤细胞移植小鼠乳腺癌模型中, SB-431542 (10 mg·kg^{-1}, i.p., 3次/周, 治疗4周) 可显著抑制乳腺癌的肺转移[52]。在小鼠结肠癌模型中, SB-431542 (每只小鼠1×10^{-4} μmol, i.p., 第3、7天各给药1次) 也可激活CTL, 诱导树突状细胞成熟, 产生抗肿瘤免疫应答[53]。该抑制剂在控制肿瘤转移、抑制组织纤维化等方面有潜在应用价值。

2.2.2 LY2109761 LY2109761是美国礼来公司研发的一种新型ALK5/TGFβRII双靶点抑制剂, 最早报道于2006年, 体外激酶抑制实验显示其对两个受体的抑制常数K_i分别为38和300 nmol·L^{-1} [54]。在细胞实验中, LY2109761可显著抑制和降低TGF-β1诱导的L3.6pl/GLT细胞迁移、侵袭和细胞中Smad2蛋白磷酸化水平。在体内实验中, LY2109761 (50 mg·kg^{-1}, b.i.d., *p.o.*, 每周一至周五给药), 与吉西他滨 (25 mg·kg^{-1}, q.d., i.p., 每周二、五给药) 联用4周治疗L3.6pl/GLT荷瘤小鼠; 单用或联用顺铂 (50 mg·kg^{-1}, i.p., 1次/周) 4周治疗裸鼠移植瘤 (卵巢癌细胞SKOV3或OV-90); 单用或联合放疗 (每次2 Gy, 第0～4天连续放疗5天; 或每次7 Gy, 第4天单次放疗) 治疗 (30～40天) 两种人成胶质细胞瘤 (U87MG和NMA-23) 的裸鼠移植瘤, 均显著降低肿瘤体积、延长动物生存期、降低自发性转移、抑制肿瘤微血管形成, 并增强放、化疗效果[55,56]。目前认为, LY2109761的双激酶抑制活性, 在抗肿瘤活性方面具有独到的优势。

2.2.3 TP-0427736 TP-0427736是一种新型ALK5激酶抑制剂, 最早报道于2013年, 体外抑酶IC_{50}为2.72 nmol·L^{-1}, 比抑制ALK3激酶的活性 (IC_{50} = 836 nmol·L^{-1}) 高约300倍。体外研究显示, TP-0427736呈浓度依赖性地抑制TGF-β1诱导的A549细胞Smad2/Smad3蛋白磷酸化 (IC_{50} = 8.68 nmol·L^{-1}), 并降低TGF-β对人毛囊外根鞘细胞的生长抑制作用。体内研究显示, TP-0427736

Table 2 Small-molecule inhibitors of ALK5 in preclinical study

Compound	Target	CAS No.	Originator	Structure
SB-431542	ALK5	301836-41-9	GlaxoSmithKline	
SB-525334	ALK5	356559-20-1	GlaxoSmithKline	
SB-505124	ALK5	694433-59-5	GlaxoSmithKline	
TP-0427736	ALK5	864374-00-5	Taisho Pharmaceutical	
LY2109761	ALK5/TGFβRII	700874-71-1	Eli Lilly and Company	
LY364947	ALK5	396129-53-6	Eli Lilly and Company	
IN-1130	ALK5	868612-83-3	SK Holdings	
SD-208	ALK5	627536-09-8	Johnson & Johnson	
R-268712	ALK5	879487-87-3	Daiichi Sankyo	
A-77-01	ALK5	607737-87-1	Japanese Foundation for Cancer Research	
A-83-01	ALK5	909910-43-6	Japanese Foundation for Cancer Research	
RepSox	ALK5	446859-33-2	GlaxoSmithKline	
GW788388	ALK5	452342-67-5	GlaxoSmithKline	

能显著抑制小鼠背部皮肤组织Smad2蛋白磷酸化, 也能显著抑制生长后期过渡至退行期引起的毛囊长度缩短, 延长毛囊的生长后期, 从而为雄激素性脱发提供一种潜在的治疗候选物[57,58]。

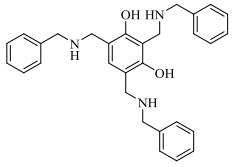
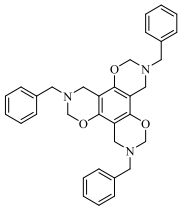
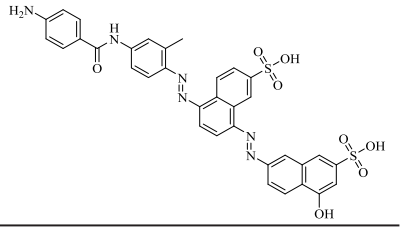
2.2.4 IN-1130 IN-1130也是一种ALK5抑制剂, 最早报道见于2006年, 其抑制ALK5磷酸化Smad3蛋白的IC_{50}为5.3 nmol·L^{-1} [59]。体外研究显示, 针对人肝癌细胞HepG2, IN-1130可抑制TGF-β诱导的Smad2蛋白磷酸化[60]。体内研究显示, 在4T1肿瘤细胞移植小鼠乳腺癌模型中, IN-1130可抑制TGF-β诱导的磷酸化Smad2蛋白入核、EMT、乳腺癌肺转移[61]。在大鼠单侧输尿管梗阻肾纤维化模型中, IN-1130可抑制肾小管间质性肾炎, 并降低磷酸化Smad2、fibronectin、α-SMA、I型胶原蛋白的表达水平, 进而显著抑制肾纤维化过程[59]。

2.2.5 其他ALK5激酶抑制剂 除上述4个代表性品种, 表2中所列其余ALK5激酶抑制剂亦作为药物候选物获得人们相应的关注。如果按照文献报道的体外抑酶IC_{50}值, 将这些分子的激酶抑制活性从高到低做一排序, 可见: R-268712 (2.5 nmol·L^{-1}) > A-83-01 (12 nmol·L^{-1}) > SB-525334 (14.3 nmol·L^{-1}) > GW788388 (18 nmol·L^{-1}) > RepSox (23 nmol·L^{-1}) > A-77-01 (25 nmol·L^{-1}) > SB-505124 (47 nmol·L^{-1}) > SD-208 (48 nmol·L^{-1}) > LY364947 (59 nmol·L^{-1})。当然, 仅依据体外抑酶活性的强弱, 并不能直接判断这些分子在细胞或整体动物水平抑制TGF-β信号通路的能力。但总体而言, 它们均不同程度对TGF-β诱导的Smad2/Smad3蛋白磷酸化、EMT、肿瘤细胞生长、毛细血管生成、组织纤维化、脱发、疤痕形成等病理过程产生良好的体内外抑制效应, 故吸引大量研发机构进行相关药用研究和评价[62-71]。

2.3 靶向TGF-β-受体相互作用的小分子抑制剂

有别于激酶抑制剂的思路, Wang等[72]采用计算机辅助药物设计, 针对TGF-β受体胞外结构域 (TGFβR-ECD) 三维结构, 基于配体/受体活性口袋的特定位点, 设计、合成了一系列结构新颖的小分子化合物, 代表性品种见表3。本课题组联合王昊课题组, 从中筛选出一类安全性较高的吲哚类化合物, 通过分子动力学模拟和表面等离子共振 (SPR) 分析, 发现该类化合物靶向结合于TGF-β1和TGFβRII-ECD。初步研究显示,

Table 3 Small-molecule inhibitors of TGF-β-TGFβR interaction

Compound	Target	Structure
NCI-48454	TGF-β-TGFβRII-ECD	
NCI-48455	TGF-β-TGFβRII-ECD	
NCI-79727	TGF-β-TGFβRII-ECD	

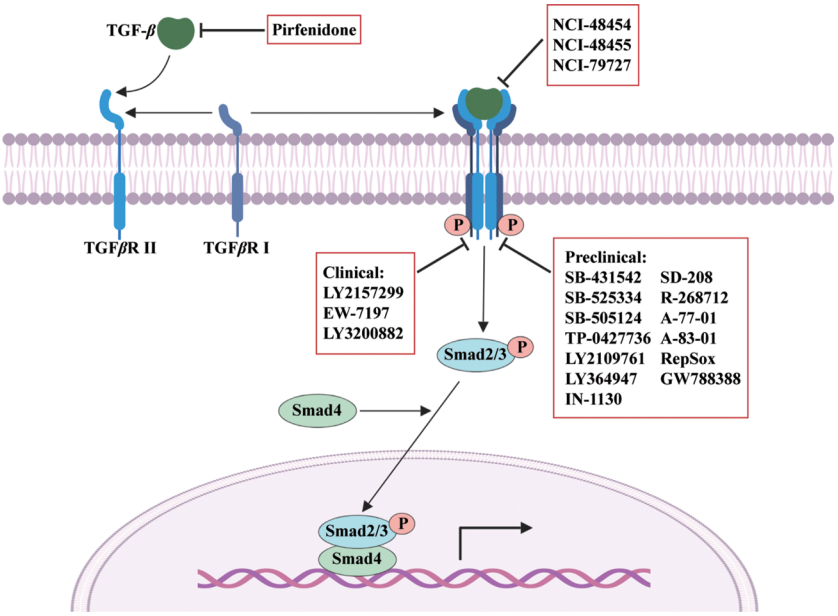


Figure 2 Small-molecule inhibitors targeting TGF-β and its receptors

这类化合物可有效阻断TGFβRI和TGFβRII的相互作用, 抑制Smad2/Smad3蛋白磷酸化及入核, 并通过相关信号通路在细胞水平抑制上皮细胞EMT过程和成纤维细胞向肌成纤维细胞转分化, 有望在体内抗肿瘤侵袭与转移、抗组织纤维化等方面发挥药理治疗作用。此类新型小分子抑制剂, 以阻断TGF-β及其受体的蛋白−蛋白相互作用 (protein-protein interaction, PPI) 起始, 进而阻断TGF-β信号转导, 其靶标特异性优于现报道的激酶抑制剂, 具有潜在应用价值。

3 总结与展望

目前, 以TGF-β及受体不同作用位点为靶, 寻找、设计、筛选各种高效、低毒新型小分子抑制剂的研究方兴未艾。如图2所示, 抑制TGF-β产生的吡非尼酮已成功上市, 而作用于ALK5激酶的LY2157299、EW-7197、LY3200882正处于临床Ⅰ期至Ⅲ期受试者募集或研究评估中。尽管临床前研究阶段的各类候选物分子仍以ALK5激酶抑制剂为主, 但以蛋白−蛋白相互作用位点为靶, 寻找TGF-β信号通路新型抑制剂的思路则颇具启发性。此外, 利用上述小分子抑制剂与其他作用机制药物合理联用, 治疗多种相关疾病也吸引着很多研究者的注意力。总之, 随着新药开发策略的日益丰富, 及安全有效小分子抑制剂的不断开发, 相信会有更多靶向TGF-β及受体的药物进入临床, 发挥应有的治疗作用。

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