

应用病毒治疗结直肠癌的研究进展*

尹磊^{①②} 孙燕来^② 综述 徐忠法^{②③} 审校

摘要 基于病毒的抗肿瘤治疗是一种新兴的生物治疗方式,病毒载体感染肿瘤组织,引起溶瘤效应,制成疫苗激活体内抗肿瘤免疫,搭载基因行癌症的基因治疗。随着对病毒的不断改造,各类病毒治疗肿瘤更趋于安全和高效,同时,病毒载体与现有的抗肿瘤疗法合理联用,可提高治疗效果。因此,基于病毒的抗肿瘤治疗将作为极具潜力的方法而逐渐引起人们的重视。本文就近年来各种病毒在治疗结直肠癌中的研究进展做一综述。

关键词 结直肠肿瘤 溶瘤病毒 病毒载体 基因治疗 免疫治疗

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Advances in virus-based therapies for colorectal cancer

Lei YIN^{1,2}, Yanlai SUN², Zhongfa XU^{2,3}

Correspondence to: Zhongfa XU; E-mail: xzf2216@126.com

¹School of Medicine and Life Sciences, Jinan University and Shandong Academy of Medical Sciences, Ji'nan 250022, China; ²The Fourth Surgical Department, Shandong Tumor Hospital, Ji'nan 250117, China; ³The Affiliated Hospital of Shandong Academy of Medical Sciences, Ji'nan 250031, China

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Abstract Virus-based anti-tumor therapies are novel biological treatments. Viral vectors can infect tumors to kill cancers directly (oncolysis), act as cancer vaccines to activate the immune system, and deliver genes with anti-tumor activity to the cancer cells. Genetic engineering has been applied to viruses to achieve more specific and efficient cancer treatment. Simultaneously, a reasonable combination of viral vectors and existing anti-tumor therapy can improve the therapeutic effect. Consequently, virus-based therapy is expected to serve as an effective anti-tumor strategy. We reviewed recent studies on the anti-tumor viral therapy of colorectal carcinoma.

Keywords: colorectal neoplasm, oncolytic virus, viral vector, genetic therapy, immunotherapy

结直肠癌(colorectal carcinoma, CRC)是世界第三大常见的恶性肿瘤,发病率呈持续上升趋势^[1]。在中国,结直肠癌5年生存率仅32%,排位死因顺位已上升到第5位^[2]。尽管结直肠癌的诊治水平不断提高,但由于其早期症状隐匿,仍有20%患者确诊时即为转移性结直肠癌^[3],且即使进行根治手术仍有50%~60%因微小转移灶而发生转移^[4]。化疗对于局部淋巴结转移者疗效有限,而在伴有远处转移者中疗效尚不肯定^[5]。远处转移、对化疗耐药、反复复发使结直肠癌患者的预后欠佳。为了提高结直肠癌的疗效及预后,人们不断深入研究结直肠癌发生及发展机制的研究,已取得不少成果。随着基因工程的发展和分子生物学的应用,利用病毒治疗肿瘤在近20年得到迅速发展,被基因改造的病毒许多已行体内外抗肿瘤疗效评估^[6]。

1 基于病毒治疗肿瘤的发展史

利用病毒治疗癌症已有100多年历史。然而其用来治疗肿瘤最初是源于偶然事件,肿瘤患者患有感染性疾病时,肿瘤出现短暂的临床缓解。1904年有报道^[7],1例白血病患者在一次流感病毒感染后病情意外好转。100多年来,病毒一直被作为实验研究对象行抗肿瘤治疗,但人们对该领域的兴趣一直波动不定。直到1991年Martuza等^[8]报道,转基因单纯疱疹病毒(herpes simplex virus, HSV)在恶性胶质瘤治疗中有一定疗效,才使基于病毒的抗肿瘤疗法日益受人关注。2005年中国国家食品药品监督管理局首次批准重组人5型腺病毒(安柯瑞)注射液用于临床治疗^[9]。至今,全世界已有200多项病毒抗肿瘤治疗的研究专利,且许多已完成临床I/II期试验。

2 溶瘤病毒直接破坏肿瘤细胞

作者单位:①山东省医学科学院,济南大学医学与生命科学学院(济南市250022);②山东省肿瘤医院外四科;③山东省医学科学院附属医院

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通信作者:徐忠法 xzf2216@126.com

溶瘤病毒(oncolytic virus, OV)治疗结直肠癌是基于病毒选择性地肿瘤细胞内大量复制最终破坏肿瘤细胞,释放子代,感染周边肿瘤细胞,在瘤体内广泛播散,而对正常组织细胞无杀伤作用^[10]。肿瘤细胞裂解后,肿瘤释放原始抗原和热休克蛋白,导致强烈的机体抗肿瘤免疫效应。另外,有些溶瘤病毒还能产生凋亡蛋白,有些可通过表达病毒蛋白或非特异性炎症反应来增强抗肿瘤免疫。NV1020是减毒的单纯疱疹病毒,删除了UL/L交界的15 kb区域和胸苷激酶基因及其启动子UL24,另外还插入了外源性TK基因,该基因受ICP4启动子的控制^[11]。NV1020 I/II期临床试验^[12]以转移性结直肠癌患者为研究对象, I期13例患者通过肝动脉注射NV1020,患者出现短暂的发热而无其他不适,认为NV1020是安全的。II期入组多线治疗后病情仍进展的患者22例,注射NV1020后,50%患者病情稳定,继续化疗后整体肿瘤控制率为68%(其中PR 1例,SD 14例),中位生存期平均延长6.4个月。因此NV1020在结直肠癌肝转移患者中具有良好疗效且不良反应小,另外,其可能通过特殊的、系统的溶瘤免疫应答逆转肿瘤的化疗耐药性,增加放疗敏感性^[13]。目前,一项NV1020联合细胞毒药物或靶向药物杀伤肿瘤细胞的大型随机II/III期临床试验正在开展。

胃肠癌腹膜转移是其常见转移形式,约超过一半患者术后复发病例为腹膜转移,患者预后不理想^[14]。Eveno等^[15]采用改造的溶瘤痘苗病毒GLV-1h153表达人钠碘转运体基因(hNIS)行体外实验,该病毒能感染复制并溶解人结直肠腺癌细胞。在小鼠结直肠癌腹膜转移模型上腹腔注射GLV-1h153后,肿瘤显著减小。另外,GLV-1h153感染的组织还可经¹³¹I SPECT扫描和荧光光学成像显示。因此,GLV-1h153不仅可用于治疗,还可用于腹膜转移病灶的诊断和评估。

对于溶瘤病毒,缺点是大部分需要瘤内局部注射,但并非所有的溶瘤病毒均如此,这种区别的原因尚不清楚。Adair等^[16]选取结直肠癌肝转移患者为试验对象,在转移病灶切除前先行呼肠孤病毒静脉注射1个周期。虽然患者在治疗前体内已有抗病毒抗体,但是血细胞中仍能检测出具有细胞毒性的呼肠孤病毒,而血浆中却未检测出,这提示细胞转运可保护病毒免受抗体破坏,使其进入肿瘤组织,发挥抗肿瘤效应。在转移病灶手术标本中,病毒蛋白在瘤细胞内表达而在正常肝组织中未表达。提示呼肠孤病毒可能被免疫细胞携带至肿瘤组织并只在瘤细胞内复制。另外,细胞载体也可保护病毒免遭体内抗体的中和,这种载体也较便捷^[17]; Mader等^[18]用自体脂

肪间充质干细胞作为麻疹病毒的载体,已被美国食品药品监督管理局批准用于卵巢癌的临床研究。

3 基于病毒载体的基因治疗

溶瘤病毒具有无限复制性和靶向性,可作为基因载体,携带外源性抗癌基因,使其在发挥溶瘤功能的同时,还可大量表达抗肿瘤基因,产生双重抗癌效应。目前,越来越多携带治疗基因的溶瘤病毒被用于抗癌研究,并取得了可喜成果^[19]。

3.1 癌基因和抑癌基因治疗

结直肠癌的发生是多基因致病的过程,通过转基因的高表达阻断肿瘤恶性转化,是控制肿瘤的有效手段之一。FHL2(four and a half LIM domain 2)是结直肠癌的一种致癌基因^[20]。Wu等^[20]以腺病毒为载体,构建了可下调FHL2蛋白表达的rAAV-shRNA-FHL2病毒,将其转染LOVO细胞株,行周期检测显示细胞停滞在G₀/G₁期,细胞生长受到抑制。ST13是一种结直肠癌特异性抑制基因,腺病毒SG500能通过hTERT、HRE启动子分别驱动E1A和E1B进行双重调节,Yu等^[21]将ST13插入腺病毒SG500中构建结直肠癌特异性腺病毒SG500-ST13,比SG500特异性更高,抗肿瘤效果更强。

3.2 免疫基因治疗

免疫基因治疗的主要策略是把免疫相关基因或细胞因子基因导入人体,增强肿瘤的免疫原性及机体对肿瘤抗原的识别和递呈能力,提高免疫效应细胞的抗肿瘤免疫功能。Zhao等^[22]使用腺病毒为载体构建表达趋化因子CCL21和细胞因子IL15基因的病毒Ad-CCL21-IL-15,在结直肠癌荷瘤小鼠行瘤内注射病毒后能显著抑制肿瘤生长。另外,经Ad-CCL21-IL-15治疗后小鼠体内能产生肿瘤特异性细胞毒T淋巴细胞免疫反应,IFN- γ 表达水平比对照组高。

3.3 自杀基因治疗

自杀基因治疗即将某些病毒或细菌所特有的前体药物转换酶基因导入肿瘤细胞,该基因能编码特殊的酶,使无毒的药物前体在肿瘤细胞内转换为细胞毒性产物,从而达到杀灭肿瘤细胞的目的。目前研究较多的有单纯疱疹病毒胸苷激酶/更昔洛韦、胞嘧啶脱氨基酶/5-氟胞嘧啶(5-FC)、硝基还原酶/硝苯亚胺。Yamada等^[23]通过表达胞嘧啶脱氨基酶的溶瘤单纯疱疹病毒阐述了病毒的复制、基因的表达和前体药物的活化之间的关系。该研究在小鼠结直肠癌模型上进行实验发现,早期加入5-FC相对于晚期加入有更大的细胞毒性;病毒瘤内注射后第6天转换5-FC成5-氟尿嘧啶(5-FU)的效率最高,治疗效果最强;此外,瘤内5-FU产物的形成未引起小鼠整体水平的5-FU升高。该研究为临床应用奠定了基础。

许多实验表明单自杀基因抑瘤效果不及双自杀基因。Boulaiz等^[24]利用逆转录病毒为载体携带凋亡素基因(apoptin)和鸟苷酸交换因子(GEF)转染DLD-1人结直肠癌细胞株,结果显示同时表达apoptin和GEF两种基因的比只表达其中一种基因的病毒载体更能促进细胞凋亡和抑制细胞活性。

3.4 抗肿瘤血管的基因治疗

肿瘤的生长和转移有赖于血管的形成,血管内皮生长因子(VEGF)促进肿瘤细胞迁移,抑制瘤细胞凋亡和诱导肿瘤新生血管。Qiu等^[25]采用慢病毒构建VEGFA靶向RNAi病毒载体感染RKO大肠癌细胞,实验结果表明抑制VEGFA能显著降低RKO大肠癌细胞的增殖、侵袭转移和肿瘤生长,其机制可能是抑制VEGFA可导致MEK/ERK-Smac/Diablo信号通路表达受阻。VB-111由非复制型腺病毒载体和修饰的小鼠前内皮素前体基因的启动子组成,其启动子通过在血管内皮细胞表达Fas的嵌合体基因导致细胞凋亡。Brenner等^[26]用VB-111在33例晚期实体瘤患者中(包括晚期结直肠癌)行剂量递增性研究发现,病毒剂量 $>3 \times 10^{11}$ VPs时患者出现自限性发热;治疗28天后疗效评价显示53%患者病情稳定;治疗后转移病灶中能检测到转基因的表达,而在血液中转基因的表达阴性。

4 基于病毒载体的肿瘤疫苗治疗

肿瘤疫苗是直接应用肿瘤抗原进行主动免疫治疗的一种产物,可增强和调节宿主免疫系统,诱导产生特异性抗肿瘤反应^[27]。早在1990年人们已开发出联合痘病毒载体表达CEA的结直肠癌疫苗。研究表明,病毒载体比传统佐剂有更强的免疫原性,其感染肿瘤组织引起炎症反应使得其成为强大的抗肿瘤免疫诱导剂^[28-29]。大部分肿瘤疫苗能激活特异性T淋巴细胞,记忆T淋巴细胞是结直肠癌的重要预后因素^[30]。另外,对于单纯疱疹病毒(herpes simplex virus, HSV)载体,机体适应的抗病毒免疫可以提高抗肿瘤免疫^[31]。

4.1 针对肿瘤特异性抗原的肿瘤疫苗

这类疫苗可以打破机体对自身抗原的耐受性。MUC1是人们发现的首个结直肠癌抗原^[32],但许多临床研究显示机体对MUC1有较强的免疫耐受性,不能诱导出MUC1特异性免疫应答^[33]。但近期研究显示处于癌前病变时的肿瘤微环境对MUC1特异性免疫具有塑造作用。Takashi等^[34]以痘病毒为载体构建携带MUC1的结直肠癌疫苗,将其注入39例晚期结肠腺癌患者中,结果发现其中43.6%患者能诱导出MUC1相关免疫反应,而另外56.4%患者未能产生免疫应答,这是因为在接种疫苗前,其体内已有高水平

的髓源性抑制细胞(MDSC),导致其免疫功能被抑制。这提示在癌前病变阶段者或免疫功能抑制者中预防性接种MUC1疫苗能避免癌前病变发展为癌症。此外,还有多种肿瘤相关抗原的病毒疫苗被用于结直肠癌靶向治疗的研究中,如p53^[35]、KSA^[36]、5T4^[37]等。

4.2 自体肿瘤疫苗

自体肿瘤细胞疫苗是使用患者自身的肿瘤细胞,在体外行放射处理后偶联免疫佐剂,再接种到患者自身。Onco-VAX是种以卡介苗为佐剂的自体肿瘤细胞疫苗,Ⅲ期临床试验显示,I~Ⅳ期结直肠癌患者肿瘤复发率减低,生存期延长^[38]。ATV-NDV是利用新城疫病毒(newcastle disease virus)感染从患者肿瘤组织分离的细胞而制成的疫苗。Schulze等^[39]将入组的结直肠癌肝转移患者随机分为对照组和接种疫苗组。肝转移病灶完整切除后,疫苗组的患者接受6次ATV-NDV治疗,结果显示接种疫苗的患者无转移生存期和总生存期显著延长。一项关于Ⅱ期或Ⅲ期结直肠癌术后残留微小瘤灶行主动特异性免疫疗法(ASI)的Meta分析显示,ASI能显著提高无进展生存期(HR=0.76, P=0.03)和总生存期(HR=0.76, P=0.007)。所有亚组患者对疫苗的副反应率为1.68%,且无显著的不良事件报道^[40]。

4.3 添加共刺激因子的肿瘤疫苗

痘病毒属转染效率高,无插入突变风险,能产生强大的免疫刺激作用^[41]。结直肠癌患者接种痘病毒疫苗rV-CEA行主动免疫,虽然观察到特异性的细胞毒T淋巴细胞反应,但客观反应率却是令人失望的^[42]。随后,痘病毒载体表达CEA和共刺激因子B7.1的肿瘤疫苗ALVAC-CEA/B7.1被研发^[43]。Ⅰ期临床试验发现晚期肿瘤患者(包括结直肠癌患者)体内能产生CEA特异性T淋巴细胞免疫,40%患者病情稳定至少达4个月^[44]。Ⅱ期临床试验应用ALVAC-CEA/B7.1联合化疗治疗结直肠癌,结果显示化疗药物并不影响ALVAC-CEA/B7.1诱导抗肿瘤免疫,有40%患者能达到客观反应,为肿瘤疫苗联合化疗的临床应用提供了支持^[45]。还有临床实验显示,表达CEA和ICAM-1或LFA-3等共刺激因子的牛痘病毒和禽类痘病毒有较好的临床疗效^[46]。目前,基于病毒载体的肿瘤疫苗能直接产生免疫反应,而无需感染肿瘤组织,有望成为新的肿瘤治疗方法。然而,基于病毒的肿瘤疫苗需要依赖完善的机体免疫,真正的肿瘤抗原特异性免疫应答还很低,这可能更适合微小转移灶的治疗。

5 展望

结直肠癌基于病毒的治疗方法有着广阔的前景,但也存在着很多障碍。一方面,肿瘤是复杂、异质性

的,肿瘤干细胞会休眠,因此溶瘤病毒难以达到理想的治疗效果;另一方面,病毒本身具有抗原性,能激活机体抗病毒免疫,病毒的进化变异会造成新的病原体感染。但随着对病毒基因和结直肠癌相关机制的了解,基于病毒治疗结直肠癌的策略也在不断完善。Pan 等^[47]利用髓源抑制细胞运载溶瘤水疱性口炎病毒,行静脉注射治疗结直肠癌转移小鼠后,小鼠生存期显著延长且无明显毒性。乏氧会造成肿瘤对放化疗产生耐受^[48]。Cherry 等^[49]针对肿瘤微环境中乏氧诱导因子(HIF)通路的激活构建新的溶瘤腺病毒 HIF-Ad 和 HIF-Ad-IL4,实验结果显示其均仅在乏氧条件下复制、表达 E1A 基因,抗肿瘤效果显著。另外,病毒在联合化疗、放疗和靶向药物治疗肿瘤时,显示无交叉耐受性,抗肿瘤效应可达到叠加或协同^[50-52]。

总之,基于病毒的抗肿瘤治疗可能将代表一种新的选择应用于临床,不同于传统的肿瘤治疗方法,病毒的复制和肿瘤的发展之间存在许多相关联的治疗路径,未来病毒治疗癌症将个体化到每位患者和每种肿瘤,而这种靶向治疗也减少病毒突变体的产生。

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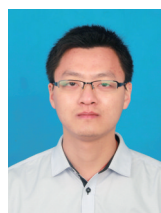
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作者简介

尹磊 专业方向为胃肠道肿瘤的外科治疗。

E-mail: 15056997829@163.com