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评 述 EB病毒发现60周年: 回顾与展望专题

EB病毒感染机制研究进展

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摘要 EB病毒属于人类疱疹病毒γ亚科, 感染全球超过90%人口. EB病毒感染每年导致数百万非肿瘤和超过20万恶性肿瘤新发病例. 人类B细胞和上皮细胞是EB病毒的主要宿主细胞. EB病毒入侵宿主细胞机制非常复杂, 需要多个病毒包膜糖蛋白与不同宿主因子协同作用决定其宿主嗜性. 近年来, 科学家们对EB病毒感染机制的研究不断深入, 为EB病毒相关疾病的精准防治提供了新思路. 本文将系统介绍EB病毒感染机制研究的现状和未来展望方向, 旨在深入理解EB病毒与宿主互作机理及干预靶点.

关键词 EB病毒, 感染, 受体, 恶性肿瘤, B细胞, 上皮细胞

EB病毒(Epstein-Barr virus, EBV)是最早被鉴定的人类致瘤病毒之一, 与多种恶性肿瘤发病密切相关, 包括B细胞来源的伯基特淋巴瘤、霍奇金淋巴瘤和弥漫性大B细胞淋巴瘤, 上皮细胞来源的鼻咽癌和约10%胃癌, 以及NK/T细胞淋巴瘤[1~3]. 此外EB病毒还与传染性单核细胞增多症, 多发性硬化症和系统性红斑狼疮等自身免疫性疾病发病相关[3~6].

EB病毒球形颗粒从外向内由病毒包膜、皮层和正二十面体核衣壳组成, 病毒衣壳包裹一条长约172 kb的线性双链DNA基因组, 表达超过80个病毒蛋白和多个非编码RNA (non-coding RNA, ncRNA)及微小RNA(microRNA, miRNA)[7~9]. EB病毒包膜上镶嵌有11种糖蛋白, 其中gp350/220, gp42, gH/gL和gB在EB病毒入侵宿主细胞过程中有关键作用[10~12]. 此外gp42在EB病毒宿主选择中具有关键作用, 高表达gp42的EB病毒容易感染B细胞, 而低表达gp42的EB病毒容易感染上皮细胞[13~15]. 和疱疹病毒科其他成员类似, EB病毒具有独特的潜伏-裂解感染生命周期, 即早期基因*BZLF1*和*BRLF1*在EB病毒生命周期转换中发挥关键作用, 影响EB病毒的感染复制、致病和传播[9].

EB病毒成功入侵宿主细胞是致病的先决条件, EB病毒主要感染人类B细胞和上皮细胞[3,12]. EB病毒使用不同的病毒包膜糖蛋白与相应受体结合入侵B细胞和上皮细胞[12,16]. 因此充分理解EB病毒感染机制将有助于科学家们开发EB病毒疫苗和靶向治疗药物. 本文将聚焦EB病毒宿主选择及相关疾病、EB病毒感染机制的最新研究进行综述.

1 EB病毒宿主选择及相关疾病

EB病毒主要通过唾液传播, 经口咽上皮和扁桃体感染组织驻留B细胞, 最终在静息记忆B细胞中形成

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EB病毒储存库[1,4,12](图1). 唾液中的EB病毒高表达gp42, 因此具有B细胞嗜性[13,14]. 然而, 唾液中的EB病毒无法直接接触口咽部驻留B细胞, EB病毒经裂解感染或转胞吞作用穿越口咽上皮细胞[17,18]. EB病毒原发感染的B细胞主要是幼稚B细胞模拟生发中心反应最终分化为记忆B细胞, 但也可能是直接感染记忆B细胞[19~23]. 在一些条件刺激下, EB病毒潜伏感染的静息记忆B细胞分化为浆细胞, 增强*BZLF1*表达, 诱导EB病毒进入裂解期, 产生并分泌子代病毒颗粒[24,25]. B细胞产生的EB病毒低表达gp42, 因此具有上皮细胞嗜性[14]. EB病毒经细胞接触或直接感染基底层上皮细胞, 上皮细胞中的EB病毒复制并分泌EB病毒颗粒至唾液中, 经密切接触方式传播给他人或再次感染B细胞维持EB病毒储存库[18,26~29].

大多数人原发感染EB病毒出现在儿童期, 表现为无症状感染; 少数人出现在青少年期, 表现为传染性单核细胞增多症[30,31]. 机体原发感染EB病毒后, EB病毒潜伏-裂解感染周期会反复出现, 表达多个EB病毒基因, 这些基因与机体正常组织蛋白有交叉抗原表位, 或抑制机体适应性免疫, 或促进炎症因子分泌, 与其他致病因子共同引发自身免疫性疾病[6,32]. 当机体出现上皮组织异型增生、系统性或局部免疫缺陷时, EB病毒将感染不易感上皮细胞, EB病毒潜伏和裂解期基因表达, 促进宿主细胞恶性转化, 并逃逸免疫监视, 最终导致恶性肿瘤发生[1,3,9,33,34].

1.1 EB病毒感染B细胞及相关疾病

EB病毒的原发感染最终在人体记忆B细胞中建立终身感染. 发展中国家人群的原发EB病毒感染多发生于儿童期, 而发达国家部分人群发生于青少年时期, 通过深吻传播EB病毒, 引起大量的B细胞增殖和强烈的T细胞应答, 导致传染性单核细胞增多症[4,31]. 两项EB病毒重组gp350疫苗临床二期实验结果显示, 靶向gp350的疫苗虽然无法清除EB病毒, 但可以减少传染性单核细胞增多症发生[35,36].

EB病毒被认为是多种自身免疫性疾病如多发性硬化症和系统性红斑狼疮的致病因素[6]. 2022年科学家们通过流行病学确定EB病毒是多发性硬化症的病

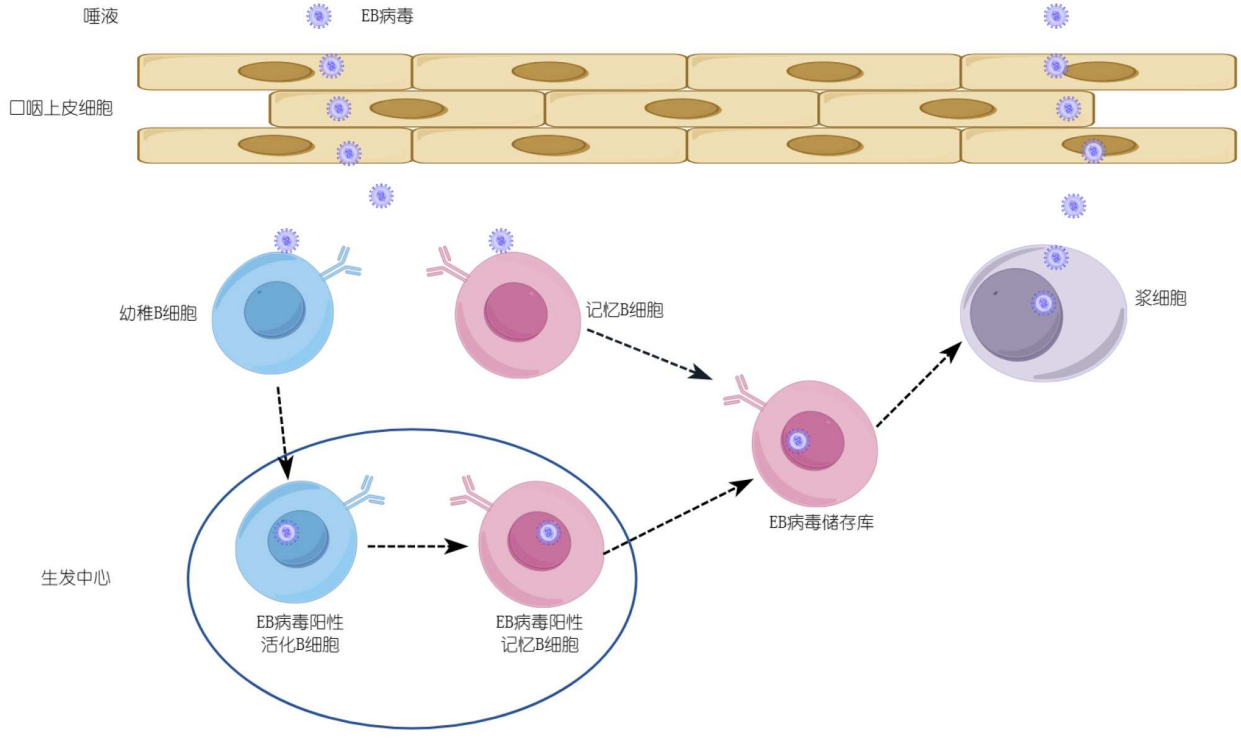

图 1 EB病毒体内感染模式图
Figure 1 Schematic showing the EBV infection *in vivo*

因[37]. 同年科学家们发现EB病毒核抗原1 (Epstein-Barr nuclear antigen 1, EBNA1)与神经元表面绝缘髓鞘上的胶质细胞黏附分子(glial cell adhesion molecule, Glial-CAM)具有交叉抗原表位, 机体体液免疫应答在清除EB病毒时也会靶向髓鞘中的GlialCAM, 从而攻击人体自身的神经系统而引发多发性硬化症[38]. 上述研究首次明确EB病毒在多发性硬化症中的致病机理, 为研究EB病毒引起其他自身免疫疾性疾病机理提供卓越的研究范本. Luftig团队基于单细胞测序技术, 在EB病毒直接感染的原代B细胞中发现, EB病毒感染可能促进一群异常记忆B细胞(atypical memory B cells, atMBCs)产生, 该群B细胞可能是多发性硬化症和系统性红斑狼疮的致病因素[23,39].

1964年科学家们通过电镜技术首次在伯基特淋巴瘤组织培养物中发现EB病毒[40]. 随后科学家们发现EB病毒可以在体外诱导原代B细胞永生化[41]. 上述研究首次证实病原体可以直接诱导人类肿瘤发生, 打开人类致瘤病毒研究的新篇章. EB病毒在静息记忆B细胞建立潜伏感染状态后, 在机体适应性免疫系统监控下, 仅表达极少量的潜伏基因[9]. 但当机体出现系统性或局部免疫缺陷时, B细胞中的EB病毒实现免疫逃逸, 表达更多的潜伏基因甚至裂解基因, 促进B细胞增殖和恶性转化, 导致B细胞肿瘤如伯基特淋巴瘤、霍奇金淋巴瘤、弥漫性大B细胞淋巴瘤、移植后淋巴细胞增殖性疾病[1,3,9].

1.2 EB病毒感染上皮细胞及相关疾病

EB病毒经唾液传播时, 首先感染的宿主细胞是口咽部上皮细胞[17]. 该过程一般无临床症状, 当被感染对象是人类免疫缺陷病毒(human immunodeficiency virus, HIV)阳性人群时, 则可导致毛状白斑[3,42].

1966年科学家们在鼻咽癌患者血清中发现高滴度的EB病毒抗体, 首次将EB病毒与上皮细胞来源的恶性肿瘤联系起来[43]. 后续研究发现鼻咽癌异型增生组织中存在EB病毒单克隆扩增, 表明EB病毒感染的窗口期早于鼻咽癌发生[44]. 目前普遍认为的鼻咽癌发病机制如下: 鼻咽组织出现癌前病变, 形成EB病毒感染窗口期, EB病毒感染鼻咽上皮细胞并表达病毒基因, 进一步促进鼻咽上皮细胞恶性转化[1,33].

约10%的胃癌与EB病毒感染密切相关, 且EB病毒阳性胃癌预后好于EB病毒阴性胃癌[45,46]. EB病毒阳性胃癌有着和鼻咽癌类似的发病机制[1,47,48]. Wallaschek等人[49]利用胃类器官发现, EB病毒受体EphA2在正常胃组织类器官中定位于细胞连接处, 而在肿瘤类器官中散在分布于整个上皮细胞膜表面使得EB病毒可以接触到EphA2, 提示癌前病变组织中EB病毒受体定位的改变产生EB病毒感染窗口期.

1.3 EB病毒感染其他细胞类型及相关疾病

1988年Sklar团队[50]首次在T细胞淋巴瘤组织标本中检测到EB病毒DNA. 随后科学家们在多种NK/T细胞淋巴瘤中检测到EB病毒, 表明EB病毒与多种NK和T细胞来源的淋巴瘤相关, EB病毒感染NK和T细胞后引起T细胞大量增殖和活化[51]. 有研究在传染性单核细胞增多症患者扁桃体T细胞中检测到EB病毒, 表明感染NK/T细胞可能是EB病毒正常生命周期的一部分[52,53].

此外, EB病毒还可能与肺淋巴上皮瘤样癌、结直肠癌、乳腺癌移植后或HIV相关平滑肌肉瘤、胆管癌和滤泡性淋巴瘤有关[54~60]. 可以肯定的是, 随着EB病毒研究的不断深入, 会有越来越多的EB病毒相关良性疾病和恶性肿瘤将被明确. 这些EB病毒相关疾病的致病机制尚不明确, 但可能与EB病毒感染后的直接恶性转化或EB病毒感染导致的适应性免疫异常导致肿瘤细胞免疫逃逸有关.

2 EB病毒入侵宿主细胞机制

EB病毒入侵宿主细胞的过程类似于其他包膜病毒, 由包膜糖蛋白介导病毒黏附、内吞和膜融合[12,61]. 相比SARS-CoV-2仅有1个刺突蛋白, EB病毒包膜上至少有7种糖蛋白在病毒入侵过程中有重要作用(表1)[10]. EB病毒入侵不同类型宿主细胞时, 使用不同的病毒糖蛋白与宿主细胞表面相应受体/辅受体结合(图2)[12].

2.1 EB病毒入侵B细胞机制

包膜糖蛋白gp350和gp42是EB病毒感染B细胞所必需的, 缺失gp350的EB病毒感染B细胞能力被极大削弱, 而缺失gp42的EB病毒则无法感染B细胞[71,72]. 缺失包膜糖蛋白gH/gL的EB病毒不影响其黏附B细胞, 但无法完成膜融合[73~75]. 包膜糖蛋白gB不影响EB病毒黏附B细胞, 但在EB病毒融合过程中是必需的[76~78].

表 1 EB病毒入侵关键糖蛋白及相应受体/辅受体
Table 1 The receptors/co-receptors of EBV entry into host cells

EB病毒糖蛋白	编码基因	受体/辅受体	靶细胞	功能
gp350/220	*BLLF1*	CD21[62] CD35[63]	B细胞和T细胞 B细胞	病毒黏附和内吞 病毒黏附
gp42	*BZLF2*	HLA-II[64]	B细胞	病毒融合
gH/gL	*BXLF2*/*BKRF2*	整合素[65,66] NMHC-IIA[67] EphA2[68,69]	上皮细胞 上皮细胞 上皮细胞	病毒融合 病毒黏附 病毒融合
gB	*BALF4*	NRP1[70]	上皮细胞	病毒内吞和融合
BMRF2/BDLF2	*BMRF2*/*BDLF2*	整合素[26]	极性上皮细胞	病毒黏附 细胞间传播

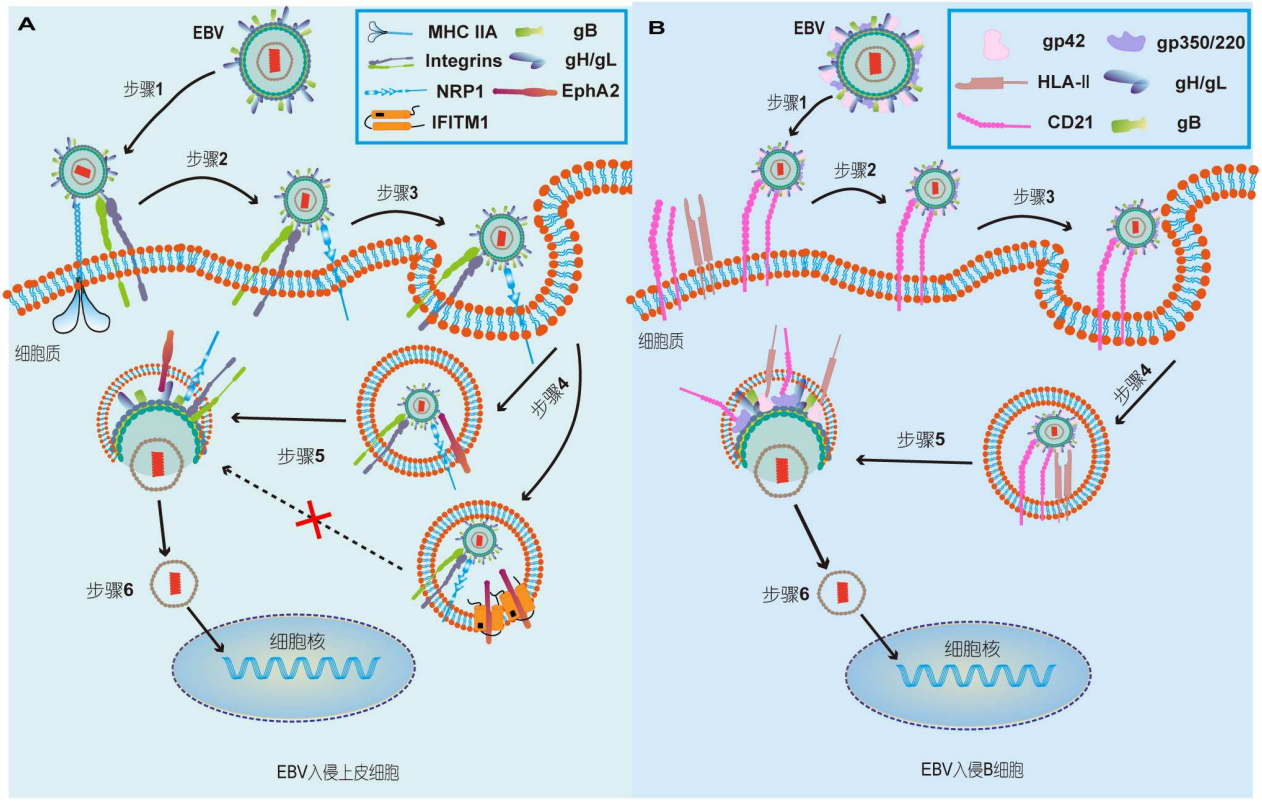

图 2 EB病毒入侵B细胞和上皮细胞示意图. A: EB病毒入侵上皮细胞示意图; B: EB病毒入侵B细胞示意图
Figure 2 Schematic showing the mechanism of EBV entry into host cells. A: The mechanism of EBV entry into epithelial cells; B: the mechanism of EBV entry into B cells

2.1.1 B细胞中EB病毒黏附和内吞

人类B细胞是最常见的EB病毒宿主细胞之一. CD21 (complement receptor 2, CR2)是最早鉴定的EB病毒B细胞受体[79]. 随后科学家们证明CD21与gp350/220结合介导EB病毒黏附和内吞进入B细胞胞质[62,80]. 此外CD21还介导EB病毒感染T细胞[81]. 2013年科学家们发现CD35 (complement receptor 1, CR1)可以代替CD21与gp350/220结合介导EB病毒感染B细胞[63]. 有研究发现EB病毒入侵原代B细胞需要经历内吞过程, 而入侵Raji等B细胞系则直接与细胞膜融合, 表明EB病毒入侵B细胞过程存在多样性[80,82].

2.1.2 B细胞中EB病毒膜融合

gp350/220在EB病毒与B细胞融合过程中可有可无[78]. gp42缺失的EB病毒可以黏附B细胞但无法完成感染, 表明gp42在进入过程中具有关键作用[72]. gp42的氨基端与gH结合, 形成gH/gL/gp42异源三聚体, gp42羧基端的C型凝集素结构域(C-type lectin domain, CTLD)与B细胞表面人类白细胞抗原Ⅱ类分子(human leukocyte antigen class II, HLA-II)结合, 引起gH/gL构象改变并导致gB暴露融合肽, gB融合肽插入细胞膜中完成EB病毒膜融合[15,83,84]. 结构生物学研究发现, gp42与HLA-II类分子结合形成“open”和“closed”构象复合物, 当形成“closed”构象时病毒与细胞膜距离被拉近, 有利于实现膜融合[85]. 近期有研究发现gp42必须经过剪切形成可溶性蛋白后才能发挥功能, 膜结合型gp42抑制EB病毒融合[86].

2.2 EB病毒入侵上皮细胞机制

EB病毒入侵上皮细胞的机制不同于B细胞, 不需要包膜糖蛋白gp350和gp42[71,87~90]. 缺失包膜糖蛋白gH/gL的EB病毒无法与上皮细胞黏附和膜融合[74,75]. 缺失包膜糖蛋白BMRF2的EB病毒与极性上皮细胞黏附能力明显降低[26,91]. 此外, BMRF2可以表达于极性上皮细胞基底面, 可能和BDLF2一起介导EB病毒在上皮组织中的细胞间传播[26,92~94]. 类似于B细胞, 在上皮细胞中包膜糖蛋白gB不影响EB病毒黏附但在病毒膜融合过程中是必需的[76~78].

2.2.1 上皮细胞中EB病毒黏附和内吞

上皮细胞通常不表达或仅表达少量CD21(或CD35), 因此EB病毒无法利用gp350/220黏附于上皮细胞表面[28,95]. 科学家们在极性上皮细胞中发现病毒包膜糖蛋白BMRF2的RGD基序与整合素β1家族结合, 介导EB病毒黏附于极性上皮细胞基底外侧膜[26,91,96]. 包膜糖蛋白gH/gL的KGD基序与上皮细胞表面整合素αvβ5, αvβ6或αvβ8结合介导gH/gL黏附于上皮细胞, 但是否影响EB病毒黏附还需要更多证据[65,66]. 曾木圣教授团队[67]利用鼻咽上皮细胞类器官模型发现非肌肉肌球蛋白重链ⅡA (nonmuscle myosin heavy chain IIA, NMHC-IIA)可以从细胞质中转位到细胞膜上, 与gH/gL结合介导EB病毒黏附于细胞表面.

1992年的一项研究利用内吞抑制剂发现EB病毒入侵人原代包皮上皮细胞不依赖于内吞途径, EB病毒结合上皮细胞受体后直接膜融合[80]. 2015年曾木圣教授团队[70]发现包膜糖蛋白gB与鼻咽上皮细胞表面神经纤毛蛋白1 (neuropilin 1, NRP1)结合激活巨内吞和脂肪依赖的内吞途径介导EB病毒入侵鼻咽上皮细胞. 上述研究表明EB病毒入侵上皮细胞过程也具有多样性.

2.2.2 上皮细胞中EB病毒膜融合

EB病毒包膜糖蛋白gH具有KGD基序, 可以与上皮细胞表面整合素αvβ5, αvβ6及αvβ8结合介导EB病毒膜融合[65,66]. 结构生物学研究发现gp42结合gH/gL后造成空间位阻阻碍整合素结合gH/gL, 因此gp42具有抑制EB病毒与上皮细胞融合的功能[15,88,97]. 2018年的一项研究发现敲除HEK293细胞中整合素αv对EB病毒膜融合没有显著影响, 表明整合素αv家族在EB病毒膜融合中的作用可能具有细胞特异性[69]. 促红细胞生成素产生肝细胞受体A2 (EPH receptor 2, EphA2)是EB病毒感染上皮细胞的重要受体, gH/gL结合上皮细胞表面的EphA2介导EB病毒膜融合[68,69]. 结构生物学研究表明EphA2的LBD结构域与EB病毒gH/gL以低亲和力互作, 而与KSHV gH/gL以高亲和力互作[98]. 近期李欣教授团队[99]发现干扰素诱导的跨膜蛋白1 (interferon induced transmembrane protein 1, IFITM1)竞争性抑制EBV糖蛋白gH/gL与EphA2的结合, 形成“钳形”竞争互作结构限制EBV的进入. 糖蛋白gB结合NRP1还促进EB病毒膜融合, 但其具体机制尚不清楚[70].

2.3 EB病毒入侵干预策略的研究进展

随着研究者更加深入理解EB病毒入侵宿主细胞机制, 为后续开发靶向EB病毒关键糖蛋白的干预策略提供重要的理论依据. 科学家们鉴定一系列靶向EB病毒糖蛋白的中和抗体, 其中有一些中和抗体针对gH/gL或gB的不同表位, 可以同时阻断EB病毒感染B细胞和上皮细胞(表2). 可以预见的是我们将来可以联合使用这些中和抗体治疗EB病毒感染相关疾病.

截止目前, 尚未有EB病毒疫苗上市. 随着纳米颗粒技术、mRNA疫苗等技术的发展, 已有多个靶向EB病毒糖蛋白的疫苗可以诱导强烈的体液和细胞免疫反应, 并在灵长类动物中产生持久的保护性抗体, 成为EB病毒预防性疫苗的强有力的候选(表3).

表 2 EB病毒入侵关键糖蛋白的中和抗体
Table 2 Neutralizing antibodies targeting the EBV glycoproteins

EB病毒糖蛋白	抗体名称	抗体的种属来源	中和抗体靶细胞
gp350/220	72A1[100]	鼠源	B细胞
gp42	F-2-1[101]	鼠源	B细胞
	5E3[102]	兔源	B细胞
	1A7, 6G7[103]	兔源	B细胞
	2B7, 2C1[104]	人源	B细胞
	A10, 4C12[105]	猕猴	B细胞
gH/gL	E1D1[106]	鼠源	上皮细胞
	CL59, CL40[75]	鼠源	上皮细胞
	AMMO1[107]	人源	上皮细胞和B细胞
	1D8[108]	人源	上皮细胞和B细胞
	769B10[109]	人源	上皮细胞和B细胞
	6H2[110]	鼠源	上皮细胞和B细胞
	10E4[102]	兔源	上皮细胞和B细胞
gB	AMMO5[107]	人源	B细胞
	3A3, 3A5[111]	兔源	上皮细胞和B细胞

表 3 靶向EB病毒糖蛋白疫苗的开发
Table 3 Vaccines against EBV glycoproteins

疫苗靶点	疫苗载体	疫苗效果	开发时间
gp350	Cowpox virus	无法阻断EB病毒感染	1995[112]
	可溶性gp350单体	减少传染性单核细胞增多症	2007[35]
	可溶性gp350四聚体体	减少传染性单核细胞增多症	2007[36]
	gp350纳米颗粒	产生EB病毒特异体液和细胞免疫	2015[113]
	gp350纳米颗粒	产生高滴度中和抗体	2021[114]
gH/gL	铁蛋白纳米颗粒	阻断小鼠体内EB病毒感染和淋巴瘤发生	2022[115]
	铁蛋白纳米颗粒	阻断EB病毒感染上皮细胞和B细胞	2019[116]
gB	纳米颗粒	猕猴体内产生持久性中和抗体	2023[117]

3 总结与展望

EB病毒在人群中感染率极高, 每年导致数百万非肿瘤和肿瘤新发病例[2,3]. 因此研发EB病毒疫苗具有巨大经济和社会价值. 然而, 之前两项gp350重组蛋白疫苗临床实验都没能阻断EB病毒感染, 其中重要原因是EB病毒采用不同的入侵机制感染人体的多种细胞类型, 靶向gp350无法阻断EB病毒感染上皮细胞[12]. 因此, 充分阐释EB病毒感染不同宿主细胞机制尤为重要.

EB病毒入侵B细胞机制较为清楚, 但入侵上皮细胞机制还有待更深入研究. 近年来曾木圣教授团队和其他课题组[12]报道整合素αvβ5, αvβ6, αvβ8, NMHC-IIA, NRP1和EphA2上皮细胞受体或辅受体. 上述受体或辅受体分别在EB病毒黏附、内吞和膜融合过程中有重要功能, 但它们如何协同促进EB病毒入侵上皮细胞机制还需更多研究. 随着冷冻电子显微镜技术的发展, 解析受体与病毒包膜糖蛋白复合物结构将有助于解答上述问题. 上述EB病毒受体或辅受体在EB病毒自然感染过程中的作用是否仍具有重要的功能尚不清楚, 将来需要借助类器官或转基因小鼠模型等技术进一步明确. 随着人源化单克隆抗体技术的成熟, 科学家们开发多个靶向EB病毒包膜糖蛋白gH/gL, gB和gp42的中和抗体, 这些抗体与糖蛋白的结合位点提示可能还有其他未被鉴定的受体.

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Research progress on mechanism of EBV infection

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Epstein-barr virus (EBV) belongs to the human herpesvirus gamma subfamily and infects more than 90% of the world's population. EBV infection causes millions of new cases of noncancer and more than 200000 new cases of cancer each year. Human B cells and epithelial cells are the main host cells of EBV. The invasion mechanism of EBV into host cells is very complex, which requires multiple viral envelope glycoproteins to cooperate with different host factors to determine its host tropism. In recent years, scientists have deepened their research on the mechanism of EBV infection, which provides new ideas for the precise prevention and treatment of EBV-related diseases. This review will systematically introduce the current status and prospects of the research on the mechanism of EBV infection.

Epstein-barr virus, **infection**, **receptors**, **malignant neoplasms**, **B cells**, **epithelial cells**

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