

组织激肽释放酶在肿瘤发展中的作用

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摘要 组织激肽释放酶(kallikrein-related peptidases, KLKs)是人类基因组中已知最大的丝氨酸蛋白酶家族。KLKs主要存在于体液和组织中,参与了广泛的生理过程,它们的异常调节与肿瘤的发生发展密切相关。该文总结了KLKs参与肿瘤发生发展的相关机制,以及部分KLKs成员在肿瘤发展进程中发挥的重要作用,综述了它们所介导的肿瘤发展进程以及作为肿瘤生物标志物和候选治疗靶点的研究进展。

关键词 组织激肽释放酶; 肿瘤; 增殖; 迁移; 侵袭; 血管生成

The Roles of Kallikrein-Related Peptidases in Tumors

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Abstract The family of KLKs (kallikrein-related peptidases) is the largest known serine protease family in human. KLKs mainly exist in body fluids and tissues, and participate in a wide range of physiological processes. Their abnormal regulation is closely related to tumor formation and development. This article summarizes the relevant mechanisms of KLKs involved in tumorigenesis and development, as well as the important role of some KLK members in tumor, and reviews the research progress of their mediation in cancer progress, and as the tumor biomarkers or candidate targets for therapy.

Keywords kallikrein-related peptidases; tumor; proliferation; migration; invasion; angiogenesis

组织激肽释放酶(kallikrein-related peptidases, KLKs)是目前人类基因组中已知最大的丝氨酸蛋白酶家族,具有胰蛋白酶活性和糜蛋白酶活性。KLKs家族最早于上个世纪30年代由KRAUT等^[1-2]从胰腺中发现,由15个保守基因(KLK1~KLK15)编码的分泌丝氨酸蛋白酶组成,它们位于染色体19q13.4区域,总长约265 Kb,在分子和蛋白质水平上,各成员间存在高度的同源性,KLKs主要存在于生物体液和组织

中,广泛参与细胞的多种生理过程,包括细胞外基质(extracellular matrix, ECM)重塑、皮肤脱屑、激素原处理和神经可塑性等。大量研究表明,KLKs的表达和调节异常与某些临床疾病(如神经退行性变、炎性皮肤病和癌症等)的发生发展密切相关^[3-5]。

近年来,肿瘤靶向治疗成为肿瘤治疗研究中的重点和热点。KLKs作为多种肿瘤的生物标志物和潜在治疗靶点,受到广泛关注。其中,KLK3现已被

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临床上广泛地用于前列腺癌的筛查、诊断、疗效评估和预后评估^[6-7],其也被称为“前列腺特异性抗原”。因此,本文就KLKs在肿瘤中发挥的重要作用进行综述。

1 KLKs的结构及活化机制

KLKs在核酸和蛋白质水平上有高度的同源性,以及相似的基因组结构。家族中所有的KLK均为单域蛋白,以不活跃的酶原形式分泌,带有一个氨基末端的信号肽,通过去除较短的前肽而被相应的蛋白酶水解激活^[8]。KLKs的信号肽(pre-region)在其分泌到胞外的过程中首先被切去,随后N-端的pro-region被水解,由此完成从无活性的蛋白酶原到有活性的蛋白酶的转变过程,该过程可由其他KLKs激活。活化后的KLKs进入乳液、唾液、精液、脑脊液、外周组织液等体液后,参与血压平衡、皮肤脱屑、精液液化、牙釉质形成和神经发育等许多重要的生理过程^[9-11]。

KLKs通过调节细胞因子和蛋白酶的活性,激活细胞表面受体或者水解细胞外基质,参与机体的正常或异常的生命活动过程。KLKs的活化是一个不可逆的过程,为了保证其活化后能够发挥正常功能,KLKs的调控受到多种因素主要包括转录、翻译水平、翻译后修饰的影响。在转录水平上其主要受到类固醇激素与KLK启动子的表观遗传修饰调控,microRNA主要参与KLK的下游效应器和转录后调控,且绝大多数KLK受到糖基化修饰^[12]。在皮肤中,白细胞介素(interleukin, IL)参与KLKs的调控,同时pH也会影响KLKs的活性。在精液的活化过程中,金属离子 Zn^{2+} 可通过抑制KLKs的活性参与精液液化过程^[2,11,13-15]。

2 KLKs对肿瘤的调控

随着对肿瘤研究的深入,肿瘤细胞被认为具有维持增殖信号,抗生长信号不敏感,抵抗细胞死亡,无限复制能力,诱导血管生成、组织浸润和转移,解锁表型可塑性,非突变表观遗传重编程,多态微生物组,衰老细胞等十四大有别于正常组织细胞的特征^[16],这些特征是现代肿瘤靶向治疗的理论基础。

丝氨酸蛋白酶主要利用丝氨酸残基催化水解肽键,在细胞生长调控、侵袭和血管生成中发挥重要作用^[17]。单个KLK或与多个KLKs共同通过与其

他蛋白酶的级联作用来促进或抑制肿瘤的进展,在肿瘤的发生发展过程中发挥了巨大作用^[5]。

2.1 促进肿瘤细胞恶性增殖

正常情况下,机体的正常组织会严格控制促生长信号的产生和释放,从而保持细胞的稳定状态,维持机体正常的结构和功能。然而,肿瘤细胞打破该稳态,通过持续增殖而不断生长,实现恶性增殖,细胞增殖失控和对生长抑制信号的逃避是肿瘤发展的关键因素。

在肿瘤细胞恶性生长的过程中,KLKs主要通过三条途径调控肿瘤细胞的增殖信号:(1)通过常规胰岛素样生长因子(insulin-like growth factors, IGFs)途径参与肿瘤的早期进展。KLKs主要通过调节关键信号元件,如胰岛素样生长因子实现调控功能。某些KLK则通过切割相应的胰岛素样生长因子结合蛋白(insulin-like growth factor-binding proteins, IGFbps),促使IGFs与相应受体结合并激活受体,进而调节细胞存活、有丝分裂和分化,如KLK2-5和14能够通过刺激癌细胞增殖的IGF1受体,参与细胞增殖调控^[18];(2)KLKs通过切割蛋白酶激活受体(protease activated receptors, PARs)的胞外N-端片段,激活下游信号通路,促进肿瘤的发生发展^[19]。PARs属于G蛋白偶联受体家族,被KLKs水解胞外片段激活后可介导致瘤信号,并激活下游的丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)^[5]。而MAPK级联通路是调节细胞增殖、分化、凋亡和应激反应等多种细胞过程的重要信号通路^[20],MAPK信号的激活可促进细胞增殖;(3)通过激肽释放酶-激肽信号促进细胞增殖。激肽是通过激肽释放酶从它们的亲代分子——激肽原的局部释放出来的。缓激肽(bradykinin, BK)是激肽释放酶-激肽系统产生的一种活性肽,通过缓激肽1受体(bradykinin receptors 1, B1R)和缓激肽2受体(bradykinin receptors 2, B2R)发挥功能。部分癌症如前列腺癌、乳腺癌和肺癌等,在很大程度上依赖于自分泌的BK信号通路来刺激肿瘤生长^[21]。

2.2 重构肿瘤细胞外基质

ECM重构是肿瘤血管生成及肿瘤转移的前提,KLKs通过水解基质金属蛋白酶(matrix metalloproteinases, MMPs)和尿激酶型纤溶酶原激活物(urokinase plasminogen activator, uPA),能够破坏生长因子储存库,调节生长因子的利用度,促进肿瘤的

转移^[22]。KLK1可激活IV型胶原酶、pro-MMP1~2和pro-MMP9, 这些酶是切割基底膜的主要成分^[23]。有研究表明, KLKs参与调控uPA的激活, 如KLK2~4和KLK12可激活uPA-uPAR系统, 并通过纤溶酶的形成促使ECM组分降解^[24-25], 纤溶酶可降解大量ECM蛋白并释放和激活促血管生长因子(vascular endothelial growth factor, VEGF)和Pro-MMPs^[26], 从而进一步促进肿瘤的转移。

2.3 诱导肿瘤中血管生成

与正常的组织一样, 肿瘤的生长也需要通过血管获得氧气与营养物质, 同时又通过血管排出二氧化碳和代谢废物以维持其正常的生理活动。在正常情况下, 机体的血管生成系统始终是保持一种相对“静止”的状态, 当人体出现外伤时或者女性处于生理周期的时候, 血管生成的“开关”会被短暂打开。而在肿瘤的发展过程中, 血管生成的“开关”会被持续激活, 不断生成新血管, 加速肿瘤对氧气和外界营养物质的摄取, 促进肿瘤的生长, 并帮助肿瘤完成转移^[27-28]。KLKs通过切割细胞外基质和基质蛋白, 如纤维蛋白、纤维连接蛋白等, 调节多种促血管生成因子的利用度, 促进血管生成^[29]。例如, 向内皮细胞中回补活性KLK12后, KLK12能够将细胞外基质或膜结合的血小板源性生长因子B(platelet-derived growth factor-B, PDGF-B)前体转化为可溶性的形式, 说明KLK12能够调节内皮细胞与基质细胞的相互作用, 释放的PDGF-B通过旁分泌的作用, 调节基质细胞分泌VEGF-A, 最终导致血管生成^[15]。同时, KLK1~2和KLK12还可裂解激肽原从而释放缓激肽受体激动剂(Lys-Des Bradykinin), 活性激肽通过上调成纤维细胞生长因子或刺激血管内皮生长因子, 刺激血管内皮细胞的生成^[30], 从而促进血管新生。

癌细胞分泌的KLK能够作用于间质, 参与旁分泌信号的传递, 诱导促血管生成环境的形成, 激活血管生成网络。其中缺氧诱导因子1 α (hypoxia inducible factor 1 α , HIF1 α)可以激活肿瘤细胞中血管生成因子的基因转录, 在非小细胞肺癌中缺氧会诱导KLK12的表达, 因其5'侧翼区含有两个HIF1 α 结合位点, 这说明在低氧环境下HIF1 α 是KLK12重要的调节因子^[31]。

2.4 调控肿瘤的侵袭和转移

KLKs通过直接或间接地重塑ECM并激活相关信号的转导, 促进肿瘤细胞的侵袭和转移^[32-33], 其主要机制与其对ECM蛋白的水解活性有关。ECM在

不同组织/细胞间构成物理屏障, ECM蛋白与整合素等细胞受体相互作用, 控制细胞运动和黏附。几乎所有的KLKs都能切割ECM蛋白, 如层黏连蛋白、纤维连接蛋白和胶原蛋白。KLK6 mRNA在结肠癌中表达上调, 在人结肠癌细胞Caco2(K-RAS突变)中进一步过表达KLK6可促进细胞的侵袭, 而沉默KLK6的表达后, 则能明显降低Caco2细胞的侵袭性^[34]。KLK13通过对ECM蛋白的水解, 促进癌细胞迁移和侵袭, 敲低高转移性肺癌细胞CL1-5中的KLK13后发现, 细胞的迁移和侵袭能力明显降低^[35]。

KLKs促迁移和侵袭作用的另一种可能机制是, KLKs对细胞连接蛋白和细胞黏附蛋白的水解。这些蛋白的减少可降低细胞黏附和细胞内聚, 减少细胞骨架组织和细胞运动, 从而促进细胞发生迁移。E-钙黏蛋白(E-cadherin)是一种跨膜钙依赖性细胞黏附糖蛋白, 是上皮标记物, 因其在侵袭性和浸润性癌症中表达降低而被称为肿瘤抑制因子^[36]。有研究表明, KLKs可调节E-钙黏蛋白基因CDH1(cadherin1)的表达, 在过表达KLK3和KLK4的前列腺癌细胞PC-3中, CDH1表达分别降低到近1/10和1/7。在胃癌和结肠癌细胞中, 过表达或活性KLK6的外源性回补增强了细胞的侵袭性, 并可观察到细胞迁移和上皮细胞间质转化(epithelial-mesenchymal transition, EMT)明显增加, 这可能与E-cadherin启动子的活性降低有关^[37]。KLKs也可能对E-cadherin产生直接影响, 外源重组KLK7直接切割PANC-1胰腺癌细胞中表达的E-cadherin, 释放可溶性片段, 减少细胞聚集, 增强细胞侵袭性^[38]。

2.5 介导化疗耐药

目前有少量研究表明, KLKs的异常表达可降低化疗药物的临床疗效, 增强化疗的不敏感性。目前临床上卵巢癌一线化疗药物主要为顺铂和紫杉醇, 有学者采用免疫组化的方法对紫杉醇化疗耐药的卵巢癌患者的癌组织进行分析, 结果显示KLK4在耐药患者病灶中高表达。随后DONG等^[39]发现内源性表达KLK4的人卵巢癌细胞系OVCA4320中和转染过表达KLK4的人卵巢癌细胞系SKOV-3后, 均可发生紫杉醇耐药。抑制KLK4可逆转其对紫杉醇的耐药, 提示KLK4可能可作为卵巢癌化疗的生物标志物。有文献报道, 在乳腺癌患者的组织中, KLK10的高表达与他莫昔芬抵抗显著相关^[40]。

2.6 参与能量代谢异常

癌细胞不仅打破了细胞增殖的正常稳态, 而且

还参与了能量代谢的异常调节,以促进细胞的增殖和分裂。WARBURG等^[41]认为,即使在有氧的情况下,癌细胞同样可以将能量代谢主要限制在糖酵解(生理情况下正常细胞仅在无氧条件下使用),对其葡萄糖代谢进行重新编程,从而产生能量,导致“有氧糖酵解”的异常状态。癌细胞可能会调节氧稳态,促进其恶性发展。KLK3在低氧的刺激下,其增强子区域的组蛋白乙酰化修饰可能会促进基因转录, HIF1 α 主要是通过组蛋白去甲基酶增加了KLK3的表达^[42]。同时, HIF1 α 还可以激活肿瘤细胞对缺氧的适应性相关基因的转录,如HIF1 α 可以与KLK3基因启动子中的缺氧反应原件结合,改变KLK3的表达水平^[43]。

2.7 调节免疫应答

KLKs在调节免疫应答方面的作用可能取决于不同细胞类型中特定的细胞表面受体的激活。KLKs主要通过调节免疫细胞和细胞因子的功能来调节免疫反应。KLKs通过诱导细胞毒性T淋巴细胞来调节抗肿瘤免疫反应,在前列腺特异性表达人KLK3的基因小鼠模型中,检测CD8⁺T细胞的表型和功能,结果显示与对照组相比,CD8⁺T细胞反应明显降低,且CD38(前列腺特异性抗原的标记)和TIM-3(T细胞耗竭的标记)的表达较高^[44]。KLKs还可能将炎性细胞因子的前体转化为其活性的可溶性形式,从而参与免疫反应。例如, KLK1具有激肽原酶活性,通过与B1R、B2R结合释放激肽受体激动剂,调节细胞因子的产生,介导免疫反应^[45]。

3 KLKs与恶性肿瘤

3.1 前列腺癌

前列腺癌是男性中常见的恶性肿瘤之一,居男性恶性肿瘤中第二位。在美国男性中,前列腺癌的发病率已经超过肺癌,成为发病率最高的恶性肿瘤。KLK3,也被称为前列腺特异性抗原(prostate specific antigen, PSA),由前列腺腺泡和管腔上皮细胞周围的细胞分泌并被释放到精液中。在临床上,血清中PSA水平的升高常被用作诊断前列腺癌的生物标志物,也可作为癌症复发的预后监测指标^[46]。精液中PSA水平与精子活力相关,提示PSA水平/活性可能影响男性的生育能力。有趣的是, GUPTA等^[47]研究发现,在875名不育男性和290名可生育男性中基因变异的KLK3与不育有关。

随着研究的深入,有学者发现在前列腺癌的样

本中KLK1、KLK4、KLK9和KLK14的蛋白表达水平显著降低,而在复发的组织样本中KLK1、KLK2和KLK14呈阴性,同时, KLK3、KLK4和KLK9的蛋白表达水平较高,其他KLKs的表达水平变化不明显^[48-49]。研究表明,在前列腺癌中, KLK2蛋白通过水解PSA的pre-信号肽激活PSA,而KLK2表达的高低与前列腺癌的分型和血清中游离的PSA相关^[50]。因此, KLK2也被认为是潜在的前列腺癌肿瘤标志物。

3.2 卵巢癌

卵巢癌是一种异质性疾病,可分为I型和II型卵巢肿瘤。虽然在组织学上有不同的亚型,但是均具有共同的分子和临床病理特征以及相似的临床病程。其中, I型肿瘤可分为低级别浆液性癌、子宫内膜样癌、透明细胞癌和黏液癌^[51], II型肿瘤包括高级别浆液性卵巢癌(high-grade serous ovarian cancer, HGSOC)、恶性苗勒管混合瘤(Malignant Mullerian tube mixed tumor, MMTT)和高级别子宫内膜样癌。尽管分类不同, I型肿瘤在疾病的早期均表现为肿瘤生长缓慢,对化疗的敏感较差,但总体预后好于II型肿瘤; II型肿瘤通常被诊断为晚期,肿瘤生长迅速,化疗敏感性高,但复发频繁,总体预后较差^[52-54]。

HGSOC是卵巢癌中最常见、最致命的亚型,约占所有卵巢癌的75%^[55]。GONG等^[56]分析了高级别浆液性卵巢癌(FIGO III/IV期)患者肿瘤组织中KLK5 mRNA的表达水平及其与临床预后的关系,发现KLK5 mRNA的高表达与晚期和较高级别有关,与化疗无效有关,且较高的KLK5 mRNA表达水平与不良的无进展生存期和总生存期有关。在晚期高级别浆液性癌中, KLK5 mRNA表达升高与术后残留肿瘤质量显著相关。DETTMAR等^[11]发现, KLK14 mRNA的低表达与卵巢癌较短的无进展生存期显著相关,与卵巢癌的特异性标志物CA125的血清水平呈负相关,因此, KLK14 mRNA的表达升高提示卵巢癌的预后良好。GONG等^[57]研究发现, KLK5和KLK7在mRNA和蛋白水平上呈显著正相关,提示这两种蛋白酶在高级别浆液性卵巢癌中协同表达。而KLK5和KLK7 mRNA表达升高均提示了晚期HGSOC的预后不良,是晚期HGSOC的预后标志物,而且KLK7比KLK5、KLK5+KLK7联合具有更强的预测价值。

同时,有研究表明,在选取的139例晚期HGSOC中, KLK9、KLK10、KLK11和KLK15 mRNA的表达与肿瘤残留肿块、腹水量无明显相关性。KLK10/

*KLK11*和*KLK9/KLK15*的mRNA之间存在显著的相关性, 其他组合之间的相关性不明显^[58]。而且单因素回归分析显示, *KLK11* mRNA的表达水平是总生存期和无进展生存期的独立预测指标, 也是HGSOC的独立预后指标^[58]。

3.3 胰腺癌

胰腺癌是对人类健康造成严重威胁的恶性肿瘤之一, 其早期症状隐匿, 不易被发现, 手术切除率低, 且极易发生转移。VLADIMIR等^[59]分析了胰腺正常组织、胰腺上皮内瘤变与侵袭性胰腺导管腺癌的组织切片中*KLK7*的表达水平, 发现癌变组织中*KLK7*的表达最高, 且在胰腺导管腺癌中*KLK7*的表达增高与预后不良密切相关。DU等^[60]研究也发现, *KLK7*在胰腺癌组织中的表达较癌旁组织高, 说明在胰腺癌组织中*KLK7*的表达异常升高; 同时降低*KLK7*在转录及翻译水平后, 胰腺癌细胞PANC-1细胞的增殖、迁移和侵袭能力明显被抑制。

*KLK7*可切割E-cadherin, 水解后的可溶性钙黏蛋白片段通过作用于ECM蛋白增强胰腺癌细胞PANC-1细胞侵袭, 减少PANC-1细胞聚集^[61]。*KLK7*在胰腺癌细胞BxPC-3中高表达, 并降低了细胞与玻连蛋白(vitronectin)的黏附, 其机制可能是*KLK7*的表达在胰腺癌中通过产生可溶性uPAR导致细胞表面uPAR断裂, 从而促进胰腺癌的转移^[62]。

3.4 乳腺癌

乳腺癌是全世界女性中最常见的恶性肿瘤, 也是一种高度异质性的肿瘤, 起源于乳腺腺体组织不受控制的细胞生长。大多数*KLKs*在正常组织或者乳汁中表达, 它们的上调或者下调往往是乳腺癌的标志^[63-65]。

最初, *KLK6*被发现转移性乳腺肿瘤细胞系中表达降低或完全失活^[66]。PAMPALAKIS等^[67]发现, *KLK6*在乳腺肿瘤中的表达降低, 并可能其以浓度依赖的方式促进乳腺癌发展。EHRENFELD等^[68]认为, *KLK6*在正常乳腺组织和基底样乳腺癌的表达显著升高, 使用缓激肽受体激动剂刺激雌激素敏感的乳腺癌细胞株, 增加体外*KLK6*的分泌, 发现乳腺癌细胞的侵袭能力增强, 提示*KLK6*可能在癌细胞的侵袭和迁移中也起着重要作用。*KLK6*可以降解细胞外基质的主要成分, 如层黏连蛋白、纤维连接蛋白和不同类型的胶原, 还与E-cadherin胞外结构域脱落有关, 从而影响细胞间的黏附和肿瘤细胞的侵袭^[69]。

相反地, 有研究报道*KLK10*在乳腺癌中起到肿瘤抑制作用, 在乳腺癌中表达下调, 其具体机制可能与高甲基化有关^[70]。

4 应用前景与展望

*KLK*靶向药物大致可以分为三类: 抑制剂(天然、蛋白质类、多肽类和小分子)、可激活的前体药物、靶向*KLK*的免疫治疗等。目前已知的主要有蛋白质类大分子抑制剂, 如丝氨酸蛋白酶抑制剂SERPIN和*KLK7*的天然抑制剂LETKI等^[71], 同时还有基于抑制或者增强*KLKs*活性的小分子抑制剂, 如1,2,4-三氮唑衍生物(1,2,4-triazole derivatives)、异甘露糖衍生物(isomannide derivatives)和吡啶咪唑啉酮衍生物1a(pyrido-imidazodiazepin-5-one)等^[72-74]。

目前研究最为深入的靶向药物主要针对*KLK3*, *KLK3*激活肽-阿霉素复合物和PROSTVAC疫苗正在进行抗前列腺癌临床试验^[75-76], 此外还有基于*KLK3*的纳米复合物前体药物的开发。同时, 还开发出基于*KLK*特殊催化位点的前列腺癌成像探针^[77], 为前列腺癌的诊断和预后判断提供了新思路。虽然*KLKs*与诸多恶性肿瘤密切相关, 但目前并没有一种*KLK*药物被成功地转化为临床。

综上所述, 随着对*KLKs*研究的深入, *KLKs*在致癌过程中发挥的重要作用日益显著。*KLKs*功能失调可导致致癌刺激的积聚, 触发肿瘤的发生, 并促进肿瘤的发展。*KLKs*是肿瘤微环境中不可或缺的元素, 除介导癌症进展和化疗耐药外, 还被作为疾病的诊断、治疗以及预后监测指标, 也是药物开发的潜在靶点。但是仍需要对*KLKs*进行深入探索: 如更加系统地研究*KLKs*与肿瘤的关系, 更好地服务临床, 聚焦*KLKs*靶向药物的开发, 以进一步提高*KLKs*在癌症早期诊断的价值和预后判断的能力, 改善癌症患者的生存率和生活质量。

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