Advances in Clinical Medicine 临床医学进展, 2021, 11(5), 2055-2061
Published Online May 2021 in Hans. http://www.hanspub.org/journal/acm
https://doi.org/10.12677/acm.2021.115294

Hans汉斯

胰腺癌循环生物标志物研究进展

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收稿日期：2021年4月7日；录用日期：2021年4月25日；发布日期：2021年5月10日

摘　要

迄今为止，胰腺癌仍是最具恶性程度的肿瘤之一，其中九成以上为胰腺导管癌(PDAC)。手术切除是能治愈胰腺癌的最佳治疗选择，但由于胰腺癌早期无明显症状，多数患者只能晚期确诊。因此，发现最具临床意义的循环生物标志物对早期胰腺癌的诊断是目前的巨大的难题。血液是最常用的生物标志物来源，具有微创、易获得、价格低廉、可重复获得和易于分析等特点，筛选最佳循环生物标志物，对胰腺癌的早期诊断具有重要的临床意义。

关键词

胰腺癌，生物标志物，早期诊断

Advances in Circulating Biomarkers of Pancreatic Cancer

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Received: Apr. 7th, 2021; accepted: Apr. 25th, 2021; published: May 10th, 2021

Abstract

Up to now, pancreatic cancer is still one of the most malignant tumors, of which more than 90% are pancreatic ductal carcinoma (PDAC). Surgical resection is the best treatment option to cure

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文章引用: 张一, 付来琳, 解祥军. 胰腺癌循环生物标志物研究进展[J]. 临床医学进展, 2021, 11(5): 2055-2061.
DOI: 10.12677/acm.2021.115294

pancreatic cancer, but because pancreatic cancer has no obvious symptoms in early stage, most patients can only be diagnosed late. Therefore, finding the most clinically significant circulating biomarkers for the diagnosis of early pancreatic cancer is a huge challenge at present. Blood is the most commonly used source of biomarkers, with the characteristics of minimally invasive, easy to obtain, low cost, reproducible and analysis, etc., screening the best circulating biomarker has important clinical significance for the early diagnosis of pancreatic cancer.

Keywords

Pancreatic Cancer, Biomarker, Early Diagnosis

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1. 蛋白质类肿瘤标志物

1.1. CA19-9

CA19-9 是目前临床唯一公认的胰腺癌循环生物标志物，CA19-9 在胰腺癌中的表达率为 70%~80%，敏感性与肿瘤分期和 Lewis 抗原有关，在 5%~10% Lewis 抗原阴性的人群中不能检测出[1]。Z. Huang 等[2]总结了 11 项关于 CA19-9 作为胰腺癌标志物的研究，共 1011 名胰腺癌患者和 1305 名健康人，结果提示 CA19-9 的 AUC 为 0.87，具有良好的诊断意义。尽管在其他非恶性胃肠疾病中，血清中 CA19-9 的水平可以升高，但其特异性随着年龄的增长而增加[3]，一项研究证明，近 2/3 的良性胆道梗阻患者 CA19-9 水平升高，而在这些患者中，血清中浓度经常达到数百或数千(U/ml) [4]，这可能由于 CA19-9 通过肝脏经胆道排除。另外，有研究发现，CA19-9 高于 300 U/ml 的值表明可能是晚期胰腺癌，并且对确定胰腺癌是否可切除和评估预后具有一定的临床意义，术后 CA19-9 水平是手术切缘阳性和肝、腹膜复发相关的独立预后因素[5]。CA19-9 也可根据肿瘤部位和血清胆红素水平选择不同临界值作为胰腺癌切除率的辅助指标，研究表示，以 100 U/ml 或 1000 U/ml 为临界值时，灵敏度分别为 68%和 41%，特异性分别为 98%和 99.8% [6]。CA19-9 除了是胰腺癌最常用的诊断标志物之一外，还能用于预测胰腺癌患者的肿瘤分期、可切除性、总生存率和治疗反应。

1.2. CEA

CEA 是一种酸性糖蛋白，最初被用作结直肠癌的生物标志物，据报道超过 60%的胰腺导管腺癌患者的 CEA 水平升高，目前临床上作为胰腺癌第二常用的生物标志物[7]。CEA 区分胰腺良恶性疾病的敏感性为 44.8%，特异性为 85.0%，其诊断效能不如 CA19-9，但如果对疑似已表现出胰腺癌体征和症状(包括上腹部疼痛，明显的体重减轻，烧心，排便习惯改变和阻塞性黄疸)的患者以 5.0 ng/ml 作为临界值，有良好的特异性，有助于临床诊断[8]。另外，CEA 在 Lewis 阴性患者中的敏感性为 63.8%，高于在总体胰腺癌的敏感性，可能作为胰 CA19-9 的补充生物标志物[9]。当 CEA 和其他临床常用指标联合后，可获得更好的临床应用价值。Gu 等人研究报道[10]，CA19-9、CA242、CA125 和 CEA 四项联合应用后，诊断胰腺癌的最终敏感性为 90.4%，特异性为 93.8%。另外，CEA 对可切除和不可切除胰腺癌的区分意义不大，但联合 CA19-9 可预测胰腺癌的可切除性[11]。

1.3. 热休克蛋白

热休克蛋白(heat shock protein, HSP)是一种具有强大功能的分子伴侣，保护活细胞免受伤害诱导，在包括癌症在内的各种疾病中都观察到热休克蛋白表达失调[12]。研究发现，HSP27 的表达水平已在多种人类肿瘤中升高，对癌症的诊断、预后或治疗有重要意义[13]。HSP27 在胰腺癌中表达上调，诊断胰腺癌的敏感性为 100%，特异性为 84% [14]。并且 HSP27 可能在对吉西他滨的耐药性中起重要作用，可作为预测胰腺癌患者对吉西他滨治疗的反应的可能的生物标志物[15]。Beatriz 等[16]研究了 57 例原发性胰腺导管癌，发现所有样本中均检测到 HSP47 的表达，并且是胰腺癌化学疗法的潜在分子靶点。Dutta 等[17]发现胰腺癌患者血清中 HSP70 升高，且在慢性胰腺炎和健康人群中筛选胰腺癌的敏感性和特异性分别为 74%和 90%，可作为检测胰腺癌的辅助生物标志物。

1.4. 其他蛋白类标记物

目前质谱技术在各种癌症生物流体和组织进行蛋白质组学中快速发展，大量研究发现了许多肿瘤标记物。Kosanam 等[18]研究了 20 例胰腺癌和 20 例胰腺良性囊肿患者中的四项血清生物标志物 DSG2、DSP、GP73 和 LAMC2，结果显示胰腺癌血清中 LAMC2 显着升高，LAMC2 的诊断能力优于 DSG2、DSP 和 GP73，并且 LAMC2 与 CA19-9 具有诊断互补性。基质金属蛋白酶 11 (MMP11)在肿瘤血管生成、迁移、存活和结缔组织降解中起关键作用[19]，既往研究发现高水平的 MMP11，提示胰腺癌预后不良[20]。近年来，许多研究证明了载脂蛋白 AII (apoAII) [21]、PhoSL-HP (岩藻糖基化斛珠蛋白蛋白) [22]、环细胞间粘附分子-1 (ICAM-1) [23]和金属蛋白酶-1 (TIMP-1) [23]等均可能成为胰腺癌的潜在生物标志物，但仍需大量的实验验证与应用。

2. 表观遗传学类标志物

2.1. miRNA

越来越多的研究表明，肿瘤细胞会向血液循环中释放大量 RNA (包括 miRNA、ncRNA 和 lncRNA)，这些 RNA 强烈抵抗血液中的 RNA 酶，血清中的水平足以进行定量分析[24]。微小 RNA (miRNA)是内源性小的单链非编码 RNA，其长度约为 22 nt [25]，可调节超过 50%的蛋白质编码基因的表达，在控制细胞增殖，分化和凋亡诱导中起重要作用[26]。miRNA 在血液循环中具有高度稳定性，体积小和丰度高的特点[27]，大量研究证明，miRNA 在胰腺癌发生过程中差异表达，可用于评估胰腺癌的诊断、预后和治疗。一项荟萃分析[28]，基于 21 个胰腺癌的 miRNA 中(包括 905 例胰腺癌和 449 例非癌血液样本)发现四个上调的 miRNA (hsa-miR-21-5p、hsa-miR-31-5p、hsa-miR-210-3p 和 hsa-miR-155-5p)和三个下调的 miRNA (hsa-miR-217、hsa-miR-148a-3p 和 hsa-miR-375)具有重大的诊断潜力，其中 hsa-miR-21-5p 具有较高的临床诊断准确性。Li 等[29]发现血清 miR-24、miR-134、miR-146a、miR-378、miR-484、miR-628-3p、miR-1290 和 miR-1825 在胰腺癌中升高，AUC 值均大于 0.7，其中血清 miR-1290 区分胰腺癌患者与对照组的能力最佳，AUC 为 0.96，并优于 CA19-9。这些血浆 miRNA 是筛选早期胰腺癌的理想生物标志物。

2.2. DNA 甲基化

DNA 甲基化是一种基因修饰，肿瘤的发生可能与 CpG 岛甲基化引起的抑癌基因失活有关，循环 DNA 可能从原发肿瘤中脱落，并且受到保护免于降解，使其成为糖核蛋白复合物[30]。最近研究发现，ADAMTS1 和 BNC1 可能作为早期诊断胰腺导管癌的生物标志物。一项研究显示[31]，诊断胰腺癌时，ADAMTS1 的敏感性为 48%，BNC1 敏感性为 79%，特异性均为 81%，BNC1 可作为新的筛查早期胰腺癌患者的生物标志物。最近研究结果显示[32]，血清 ADAMTS1 诊断胰腺癌的敏感性为 87.2%，特异性

为 95.8%; BNC1 敏感性为 64.1%，特异性为 93.7%; 联合两者后敏感性可达到 97.4%，特异性增至 91.6%，具有良好的诊断价值。Li 等[33]发现八个无细胞 DNA 甲基化可以将胰腺癌患者与健康人区分开，包括 TRIM73，FAM150A，EPB41L3，SIX3，MIR663，MAPT，LOC100128977 和 LOC100130148。此外，MUC2 [34]、SEPT9 [35]、p16、RARβ 和 TNFRSF10C [36]等在也促进胰腺癌的发生和发展。DNA 甲基化影响基因表达而不影响 DNA 序列，表观遗传变化发生在肿瘤发生的最早阶段，因此为早期胰腺癌提供了检测和诊断疾病的新方法，血清 DNA 甲基化状态的测定具有早期发现胰腺癌的强大潜力。

3. 外泌体

外泌体是一种大小约 50~150 nm 的许多细胞分泌的囊泡细胞器，它们从健康细胞和癌细胞中脱落到血液和其他体液中，参与信号转导、免疫应答、肿瘤发生、发展、侵袭和转移等一系列生理和病理过程[37]。glypican-1 (GPC1)蛋白是由 GPC1 基因编码，在许多癌症组织中高度表达，包括 PC、卵巢癌和胶质瘤等[38]，下调 GPC1 蛋白的表达可以抑制癌细胞的生长，对胰腺的早期诊断有一定作用[39]。Dong [40]等人，GPC1 对 76 例胰腺癌患者的诊断效果，结果提示 AUC 为 0.885。另外，GPC-1、CA19-9 和 EUS-FNA 的联合可以进一步将诊断胰腺癌的准确性提高到 84% [41]。Plectin-1 是一种大的 500 kDa 蛋白，仅与线粒体有关，可以结合特定的肽，胰腺癌中表达上调，可能有助于检测癌前病变和胰腺癌[14]。此外，CKAP4 与外泌体一起从胰腺癌细胞中释放，在胰腺癌血清中水平高于健康对照组，有作为胰腺癌生物标志物的潜力[42]。另一项研究发现[43]，来自外泌体的原癌基因间充质－上皮转化因子(c-Met)诊断胰腺癌的灵敏度为 70%，特异性为 85%，且 c-Met 阳性患者的术后生存时间显著缩短。另外，许多来自外泌体，如 miR-21，miR-155，miR-17-5p 和 miR-196a，miR-10b 和 miR-1246 等，均可作为胰腺癌的循环生物标志物[44]。

4. 循环肿瘤细胞

循环肿瘤细胞(circulating tumor cells, CTC)是指从原发肿瘤脱落进入脉管系统的恶性细胞，胰腺癌患者所有阶段的血液中均能发现 CTC，以及 CTC 的存在与存活率差之间有明显关联，与没有 CTC 的患者相比，具有 CTC 的患者预后更差[45]。CTC 目前已获得美国食品药品监督管理局(FDA)的批准，可作为许多癌症治疗过程的辅助性预后指标[46]。Colin 等[47]，采用 NanoVelcro/LCM 对 12 例胰腺癌患者的单个 CTC 的 KRAS 突变测序，发现 100%患者存在的 CTC，并证实了 92%的患者中至少一个 CTC 中存在 KRAS 突变。有学者在胰腺上皮内瘤变的小鼠模型中发现了 CTC，这表明在胰腺癌的早期阶段已有 CTC 存在，这意味着 CTC 可能作为胰腺癌早期诊断标志物[48]。但由于目前检测技术限制，难以应用于临床，随着技术的发展，CTC 有望成为胰腺癌的循环生物标志物。

5. 炎症因子

炎症因子包括趋化因子、生长因子和细胞因子。目前越来越多的证据表明白介素(interleukin, IL)是胰腺癌的潜在生物标志物。Feng 等[49]检测 68 例胰腺癌患者的血清 IL-1b、IL-2、IL-6、IL-8、IL-10、IL-13、IL -15 和 IL-23 的水平，结果显示 IL-6、IL-8 和 IL-10 的水平与预后明显相关。联合 IP-10、IL-6、PDGF 和 CA19-9 区分胰腺癌和良性疾病的能力优于 CA19-9 [50]。另外，血清 CRP、IL-6、可溶性肿瘤坏死因子受体 II 型(sTNF-RII)和巨噬细胞抑制性细胞因子 1 (MIC-1)水平与胰腺癌患者生存呈负相关[51]。胰腺癌患者的血清 CXCL8 水平显着高于慢性和急性胰腺炎以及其他消化系统肿瘤患者，诊断能力可能优于 CA19-9 和 CEA [52]。此外，血清 CXCL-16 和 IL-1B 有更好的预测胰腺癌恶病质的能力[53]。亮氨酸 α2-糖蛋白-1 (LRG-1)是人血清中的一种炎症蛋白，最近的研究表明，胰腺癌患者的血清 LRG-1 水平升高[54]。这些发现证明了炎症因子对诊断胰腺癌的意义。

6. 总结与展望

胰腺腺癌是一种致命疾病，由于起病隐匿，早期诊断困难，只有极少数患者可以被治愈，仍缺乏真正有效用于筛查该疾病的临床有用标记物，早期诊断仍是世界难题。CA19-9、CEA 和 CA125 是目前常用的血清学标志物，但敏感性和特异性欠佳，早期诊断价值更差，漏诊或误诊率较大。目前越来越多新型生物标志物的涌现(如 miRNA、DNA 甲基化和外泌体等)，成为研究热点，但目前仍没有能应用于临床的最优选择，未来需要大量实验去比较、验证和确认。随着相关技术的成熟与完善，未来可能会发现更灵敏、特异性更高的循环生物标志物以发现早期胰腺癌。

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