综述

恶性间皮瘤诊断相关循环生物标志物的研究进展

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摘　要:恶性间皮瘤是一种原发于浆膜表面,侵犯胸膜、腹膜、鞘膜和心包的恶性肿瘤,由于缺乏特异的临床症状,只有不到5%患者能在早期确诊。因此,亟需寻找安全有效简便的早期诊断方法。目前,蛋白及相关代谢产物如可溶性间皮素相关肽、巨核细胞促进因子、骨桥蛋白等已经得到广泛研究。近年来,蛋白质组学的兴起,人们相继发现了纤蛋白-3、高迁移率分组盒1等潜在的生物标志物。同时,基因组学研究发现微小RNA,如miR-126、miR-103、miR-92a等,作为稳定的内源性物质常在癌症中失调,可作为检测恶性间皮瘤的重要生物标志物。全文从蛋白质及代谢产物、基因组学两方面入手,介绍其在恶性间皮瘤中的特点及现状,并就近年来研究的有应用前景的循环生物标志物进行综述。

主题词:恶性间皮瘤;循环生物标志物;微小RNA;蛋白质组学
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Research Progress on Circulating Biomarkers for Diagnosis of Malignant Mesothelioma

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Abstract: Malignant mesothelioma is a malignant tumor primarily found on the serosal surface, which can invade the pleura, peritoneum, tunica vaginalis and pericardium. Due to lack of specific clinical symptoms, less than 5% of the patients can be diagnosed early. Therefore, a safe, effective and simple method for early diagnosis is urged. Circulating biomarkers are rich in types and can be used as non-invasive diagnostic method with great value. At present, proteins and related metabolites such as soluble mesothelin related proteins, megakaryocyte promoting factor, osteopontin have been extensively studied. In recent years, the rise of proteomics has led to the discovery of potential biomarkers such as fibrin-3 and high mobility group box 1. At the same time, the genomics studies have found that microRNAs, such as miR-126, miR-103 and miR-92a, as stable endogenous substances, are often maladjusted in cancer and can be used as important biomarkers for the detection of malignant mesothelioma. This article introduces the characteristics of proteins, metabolites and genomics in malignant mesothelioma, and summarizes the promising circulating biomarkers of mesothelioma studied in recent years.

Subject words: malignant mesothelioma; circulating biomarker; microRNA; proteomics

恶性间皮瘤是一种较为少见的原发性肿瘤,约80%为恶性胸膜间皮瘤[1],男性好发于65岁以上人群。恶性间皮瘤是一种职业性肿瘤,其发病与石棉接触史有关。尽管越来越多的国家禁止使用石棉,但由于从石棉接触到恶性间皮瘤发病大约10~40年的潜

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伏期[2],所以,全球范围内恶性间皮瘤发病率仍将持续增加。大多数工业化国家恶性间皮瘤的死亡率每年增加约5%~10%[1]。恶性间皮瘤发病隐匿,常以胸痛、胸腔积液为初始就诊原因,缺乏特殊症状,导致诊断延误,患者确诊时常已晚期,确诊后的中位生存时间约1年[1],现有治疗只能缓解病症,无法治愈。尽管作为诊断恶性间皮瘤金标准的病理活检的灵敏度较高[3],但是此方法为侵入性操作且依赖患者的身体状况,也不适合作为早期诊断方法。因此,寻找有效的合适的生物标志物进行恶性间皮瘤的诊断非常有必要,操作简便、费用经济、非介入性的循环生物标志物检查对于早期诊断尤为重要。

一个好的生物标志物应该有一些重要的特征,比如侵袭性小,可在血液、胸腔积液等易于获得的生物液体中测量;特异度高,以避免在健康受试者中出现假阳性结果;灵敏度高,减少漏诊、误诊率;能区分健康人和患者以及不同病理类型之间的差异[4]。根据目前的研究,鉴别诊断恶性间皮瘤常见的生物标志物包括可溶性间皮素相关肽、巨核细胞促进因子、骨桥蛋白等。现在还有越来越多的组学研究,包括基因组学、蛋白质组学、代谢组学研究方法等也用于恶性间皮瘤标志物的确定。

1 蛋白及代谢产物标志物

目前经典的蛋白质及代谢产物生物标志物有可溶性间皮素相关肽、巨核细胞促进因子、骨桥蛋白等标志物。随着蛋白质组学等检测方法提升,越来越多的潜在循环生物标志物陆续出现,如钙网蛋白、纤蛋白-3、高迁移率分组盒1、半乳糖凝集素1、表皮生长因子受体、Syndecan-1、基质金属蛋白酶-7、细胞程序性死亡配体1、血管表皮生长因子、载脂蛋白CI、CA125、Cyfra 21-1等。通常采用蛋白质组学分析和酶联免疫吸附实验来检测。蛋白质组学是生物体和系统在一定时间内,在不确定的生理、病理条件下,表达出的一整套蛋白质,是一种产生蛋白质特征的高通量方法,最近被用于有效筛选生物标志物,提高不同癌症的诊断准确性[5]。由于样本量较少,目前使用蛋白质组学研究恶性间皮瘤仍受到一定的限制,但总的来说,蛋白质组学是一种极有发展前景的恶性间皮瘤的诊断工具。

1.1 可溶性间皮素相关肽

间皮素是恶性间皮瘤研究广泛的生物标志物之一,其在正常细胞中低水平表达,在正常组织中不可检测,但在恶性间皮瘤、胰腺癌、卵巢癌、肺癌和其他癌症中大量表达。经过一系列表达,间皮素可在血清中生成可溶性间皮素相关肽 (soluble mesothelin-related peptides,SMRPs)。SMRPs是唯一经美国食品药品监督管理局 (Food and Drug Administration,FDA)批准的恶性间皮瘤生物标志物[6]。SMRPs水平可以用来鉴别未接触石棉者、接触石棉者和良性胸膜疾病患者。需要注意的是,血清间皮素水平在肾功能损伤的患者中也会升高。因此,在无肾脏疾病的情况下,SMRPs水平的升高应引起恶性肿瘤的怀疑。同时,SMRPs水平升高有利于对有恶性间皮瘤风险的胸腔积液患者进行临床诊断,胸腔积液SMRPs的升高比血清SMRPs升高更敏感。

1.2 巨核细胞促进因子

间皮素可分裂得到巨核细胞促进因子(megakaryocyte promoting factor,MPF),与SMRPs相似。研究证明恶性间皮瘤患者血清MPF水平高于健康受试者、与石棉相关的良性疾病患者和肺癌患者。但由于现有研究对象的样本量较小,不足以证明MPF可以作为诊断恶性间皮瘤的生物标志物,需要今后大范围的进一步研究验证[7]。

1.3 骨桥蛋白

骨桥蛋白是一种带负电的非胶原性骨基质糖蛋白,多数存在于各种组织和细胞中。骨桥蛋白在生物循环中发挥重要作用,比如细胞-基质相互作用、免疫调节、肿瘤发生和细胞迁移。研究表明,骨桥蛋白在暴露于石棉的细胞中过度表达,在石棉诱发致癌的动物模型中也过度表达[8]。

1.4 钙网蛋白

研究表明,浆液性积液中的钙网蛋白在区分恶性间皮瘤和转移性癌中存在一定作用,但该结果仍有争议[9]。

1.5 纤蛋白-3

纤蛋白-3(fibulin-3,Fb-3)是一种糖蛋白,由含有纤维蛋白样细胞外间质蛋白1的表皮生长因子编码,在多数正常组织中少量表达,对调节细胞增殖和迁移起重要作用,特别是与肿瘤发生有关。Kirschner等[10]研究发现,恶性间皮瘤患者的血浆Fb-3水平明

显高于仅接触石棉的患者。恶性间皮瘤患者的胸腔积液中 Fb-3 水平显著性高于其他胸腔积液患者。Fb-3 是否有望成为恶性间皮瘤患者的生物标志物还需要进一步验证。

1.6 高迁移率分组盒 1

一系列研究表明，高迁移率分组盒 1(high-mobility group box 1,HMGB1）在石棉暴露诱导后被释放,参与介导免疫反应,同时能诱导细胞发生上皮间质转化。因此,有学者猜测 HMGB1 在恶性间皮瘤的发生发展过程中起到关键作用。相关研究发现,与对照组相比，石棉暴露组患者血清 HMGB1 表达水平明显升高；在恶性胸膜间皮瘤患者和弥漫性恶性腹膜间皮瘤患者中，血清 HMGB1 水平明显高于良性石棉相关疾病和接受过石棉暴露的健康人。因此,在有石棉暴露的高危因素的人群中,HMGB1 有望成为诊断恶性间皮瘤的生物标志物[11]。有学者发现,恶性胸膜间皮瘤患者的高乙酰化 HMGB1 水平明显高于石棉暴露患者和健康对照组。在临界值为 2.0 ng/mL 时,血清高乙酰化 HMGB1 区分恶性胸膜间皮瘤患者与石棉暴露个体和健康对照的灵敏度为 100%。因此,高乙酰化 HMGB1 可能作为鉴别恶性胸膜间皮瘤患者潜在的诊断标志物[2]。

1.7 半乳糖凝集素 1

半乳糖凝集素 1(Galectin 1)可以诱导肺癌 T 细胞凋亡,抑制免疫系统,增加侵袭和转移。有研究在上皮样恶性间皮瘤患者胸腔积液蛋白质组学中进行筛选，并与肺腺癌患者的胸腔积液蛋白质组学进行比较，发现 Galectin 1 是恶性胸膜间皮瘤的预测因子。Galectin 1 对恶性胸膜间皮瘤的鉴别能力似乎等于或大于间皮素。Galectin 1 被认为是用于间皮瘤诊断的临床验证的候选指标。Javadi 等[12]比较恶性胸膜间皮瘤患者(n=42)和良性样本(n=40)胸腔积液中 Galectin 1 表达水平，发现恶性胸膜间皮瘤患者胸腔积液中 Galectin-1 的表达水平显著性高于良性渗出液。因此,Galectin 1 也可用于鉴别恶性胸膜间皮瘤与良性胸腔积液。

1.8 表皮生长因子受体

表皮生长因子受体(epidermal growth factor receptor,EGFR）是表皮生长因子受体家族成员之一。表皮生长因子受体家族在细胞生理过程中发挥重要的调节作用。EGFR 广泛分布于哺乳动物上皮细胞、成纤维细胞、胶质细胞、角质细胞等细胞表面,EGFR 信号通路对细胞的生长、增殖和分化等生理过程发挥重要的作用。研究发现恶性间皮瘤始终表现出 EGFR 的高表达[13]。

1.9 Syndecan-1

Syndecan-1(CD138,SDC-1)是硫酸乙酰肝素蛋白多糖家族的成员,主要存在于细胞膜上。SDC-1 脱落可以增强 EGFR 磷酸化和下游信号通路，调节不同的细胞过程,包括增殖、迁移、分化和血管生成,从而抑制或促进肿瘤的进展。Javadi 等[12]通过实验发现，恶性胸膜间皮瘤患者胸腔积液中 Syndecan-1 水平明显高于良性患者。

1.10 基质金属蛋白酶-7

基质金属蛋白酶（matrix metalloproteinase,MMP)被认为是最重要的蛋白酶家族,帮助 Syndecan-1 的胞外区域从细胞表面释放出来,转化为细胞表面受体的竞争激活剂或抑制剂,介导肿瘤进展的中心事件，包括侵袭、转移、血管生成和细胞存活[14]。Davidson 等[15]研究发现,基质金属蛋白酶-7(matrix metalloproteinase-7,MMP-7)对恶性间皮瘤和恶性浆液性癌鉴别诊断的灵敏度和特异度分别为 46%和 100%。MMP-7 具有高度的特异度,但仅具有中等的灵敏度,可作为鉴别良恶性间皮细胞癌的诊断依据。

1.11 细胞程序性死亡配体 1

细胞程序性死亡配体-1（programmed death-1 ligand 1,PD-L1)蛋白在抗肿瘤免疫反应中起中心作用。Carosio 等[16]评估了 84 例恶性胸膜间皮瘤患者胸水中 PD-L1 可溶性形式(sPD-L1)的基线表达水平,并将其与匹配肿瘤中 PD-L1 状态和患者的总生存率(overall,survival,OS)相关联,发现 sPD-L1 在所有胸水中都有不同程度的表达。在非上皮细胞亚群中,sPD-L1 水平略高，表明 sPD-L1 水平可能参与恶性胸膜间皮瘤组织类型的不良预后。与 PD-L1 阴性的肿瘤患者相比,sPD-L1 含量在 PD-L1 阳性肿瘤患者中往往更高,并且 sPD-L1 浓度与 OS 呈正相关。

1.12 血管表皮生长因子

血管表皮生长因子(vascular epidermal growth factor,VEGF)是胚胎发生和肿瘤生长过程中血管生成的关键信号分子。研究发现间皮瘤患者的胸腔积液中 VEGF 水平与 Syndecan-1 水平相关，并具有预后价值[8]。

综上,目前对恶性间皮瘤的诊断标志物的研究越来越得到重视。随着研究技术的不断革新,近年来关于恶性间皮瘤相关研究快速出新(Table 1)[7-9,11-13,15-31],但由于缺乏有力的实验证据或者样本数少,限制了其在临床中的应用,还需进一步的研究。

2 基因组学生物标志物

微小 RNA(miRNA,miR)是内源性、非编码的 RNA。研究表明,miRNA 广泛存在于人的循环组织和体液中,其含量高低对人体多种疾病具有诊断价值[32]。Han 等[33]研究发现,组织和体液中的 miRNA 作为恶性胸膜间皮瘤的诊断标志物,其准确性尚不令人满意。但是,循环 miRNA 具有稳定性、非侵入性、易检测、经济等特点,同时某些 miRNA 在癌症的发展进程中有差异性表达。因此,miRNA 有望成为人类癌症诊断的重要标志物。与恶性间皮瘤相关的循环 miRNA 研究较少,而且由于 miRNA 分析方法和所采用的技术方法的不同,需要大规模、标准化的验证研究来评估临床相关性,才能最终从实验室走向临床应用。

近年来发现的与恶性间皮瘤有关的 miRNA 研究(Table 2)[32,34-42],表明循环 miRNA 可能是诊断恶性胸膜间皮瘤的理想候选者。循环 miRNA 是生物信号的细胞外信使,参与肿瘤与其周围微环境之间的通讯,具有稳定性、器官和组织特异性。监测循环中相关 miRNA 的动态变化能够早期并敏感地识别疾病临床前阶段、区分肿瘤转移和潜在的恶性病变。因此,反映生理和/或病理条件的循环 miRNA 图谱[32],有可能克服迄今为止单个血液生物标志物的局限性,并可能对早期癌症检测产生重大影响。现有研究支持 miRNA 诊断恶性间皮瘤的可能作用,目前已经发现了一系列与疾病关联性较强的 miRNA,并提示其可能为该疾病的诊断及预后的标志物。

目前,蛋白质及代谢产物、miRNA 是恶性间皮瘤诊断中最常用的标志物。许多传统的蛋白质及代谢产物作为恶性间皮瘤的生物标志物具有一定的特异度和灵敏度,例如 SMRPs、MPF、OPN、Fb-3、HMGB1 等,运用特定方法检测其在循环中含量的异常变化可以协助区分恶性间皮瘤和其他胸膜类疾病,但是不能独立诊断,同时对于阴性结果的患者也无法排除恶性间皮瘤的可能。miRNA 是肿瘤与其周围微环境之间通讯产生的生物信号的细胞外信使,是稳定的、器官和组织特异性的、能够在疾病的临床前阶段识别癌症,以及区分肿瘤转移和潜在的恶性病变。例如 miR-26b、miR-25、miR-101、miR-126、miR-103/miR-103a-3p、miR-16、miR-17、miR-197-3p、miR-32-3p 等。然而,非编码 RNA 数量极其庞大,还包括 lncRNA、circRNA、siRNA 和 piRNA 等,相关研究已经证明循环中的这些非编码 RNA 含量与恶性胸膜间皮瘤的发生、发展及预后存在一定的联系。由于恶性间皮瘤属于罕见肿瘤,又有较多病例被误诊为其他胸膜或肺部疾病,目前恶性间皮瘤的临床样本极少;同时蛋白、代谢产物、miRNA 等物质在循环中的改变又较细微,检测方法复杂,以上问题限制了恶性间皮瘤相关循环生物标志物研究的展开深度,要将目前发现潜在的生物标志物正式应用于临床还有较长的路要走。今后对恶性间皮瘤临床诊断的早期生物标志物研究需要联合生物信息学、基础实验、代谢物检测和临床试验等多学科多中心进行更积极的探索。

Table 1 Protein and metabolite markers

Proteins and metabolites	Origination	Meaning	Reference
Soluble mesothelin-related peptides (SMRPs)	Serum and pleural effusion in 74 mesothelioma, 35 patients with pleural metastasis of cancer, and 28 patients with benign pleural lesions associated with asbestos exposure	Scherpereel, et al. The area under the subject operating characteristic curve (AUC) of serum SMRPs used to distinguish mesothelioma and benign lesions was 0.872(sensitivity=80%, specificity=82.6%). The AUC of serum SMRPs for differentiating metastasis and mesothelioma was 0.693 (sensitivity=58.3%, specificity=73.3%). The SMRPs in pleural effusion was higher than that in serum in all groups	[17]
	13 studies were included in meta-analysis	Gao, et al. Summarized 13 studies and summarized the results of SMRPs in the diagnosis of mesothelioma in pleural effusion. The summary analysis showed that the sensitivity was 68% and the specificity was 91%. The summary AUC area was 0.75. Subgroup analysis showed that the AUC of the cohort group with histological diagnosis could be increased to 0.86	[18]

(Continued)Table 1 Protein and metabolite markers

Proteins and metabolites	Origination	Meaning	Reference
Soluble mesothelin-related peptides (SMRPs)	217 patients with stage Ⅰ or Ⅱ epithelioid, bipolar mesothelioma and 1612 patients with symptoms or high-risk control group	Hollevoet, et al. Included 16 studies for meta-analysis, summarized the data results, and evaluated the application of mesothelin in early diagnosis. The result area under the ROC curve was 0.77. The specificity was 95% and the sensitivity was 32%	[19]
	44 patients with mesothelioma, 169 patients with other tumors or inflammatory lung disease or pleural disease, and 28 controls (non asbestos exposed patients)	Robinson, et al. The concentration of SMRPs increased in 37(84%) of 44 patients with mesothelioma, 3(2%) of 160 patients with other cancers or other inflammatory lung diseases or pleural diseases, and there was no increase in the concentration of SMRPs in 28 controls	[20]
Megakaryocyte promoting factor(MPF)	101 healthy controls, 46 patients with benign respiratory diseases, 89 healthy asbestos contacts, 123 patients with benign asbestos related diseases, 63 patients with lung cancer and 85 patients with mesothelioma	Serum SMRPs and MPF levels could distinguish mesothelioma patients from other cohorts(P<0.001). In addition, no significant difference was found between SMRPs and MPF (SMRPs=0.871, MPF=0.849; P=0.28). Further studies confirmed the equivalent diagnostic performance of SMRPs and MPF in distinguishing mesothelioma from other diseases	[7]
Osteopontin , OPN	7 studies were included in meta-analysis	The combined sensitivity was 57% and the specificity was 81%. The data suggested that OPN may be a useful diagnostic marker of mesothelioma, but due to the small sample base, larger studies are needed to confirm these findings	[21]
Calretinin	Serum or tissue of 163 patients with mesothelioma and 163 controls	Johnen, et al. Calretinin can detect all major subtypes except sarcomatoid mesothelioma. After excluding sarcomatous mesothelioma, the sensitivity of calretinin was 71%. Combined with calretinin, the sensitivity of mesothelin was increased from 66% to 75%	[9]
	18 studies were included in meta-analysis	Sensitivity and specificity of calretinin in the diagnosis of mesothelioma were 91% and 96%. The results showed that calretinin may be a useful diagnostic tool for the diagnosis of mesothelioma	[22]
Fibulin-3 (Fb-3)	Plasma of 92 mesothelioma, 136 non neoplastic asbestos contacts, 93 non mesothelioma exudates and exudate of 43 healthy controls, exudates of 7 mesothelioma, 39 benign patients and 54 other tumors	Pass HI, et al. Level of Fb-3 did not change with age, gender, asbestos exposure time or imaging changes. The level of Fb-3 in mesothelioma patients was significantly higher than that in asbestos exposed patients without mesothelioma(P<0.001). The level of Fb-3 in exudate of patients with mesothelioma was significantly higher than that of patients without mesothelioma(P<0.001). It had 96.7% sensitivity and 95.5% specificity for early mesothelioma combined with asbestos exposure	[23]
	Serum and pleural effusion in 9 patients with benign pleural effusion and 25 patients with mesothelioma	Creaney, et al. Fb-3 was significantly increased in serum and pleural effusion. The sensitivity of serum Fb-3 to mesothelioma was 100%, 78% to non malignant exudate, 82% to malignant exudate caused by metastatic disease, and 88% to mesothelioma	[24]
	7 studies were included in meta-analysis	Combined sensitivity and specificity of Fb-3 in distinguishing mesothelioma and cancer-free individuals were 62% and 82%, corresponding to AUC of 0.81	[25]
High mobility group box 1 (HMGB1)	Serum from 13 patients with diffuse malignant peritoneal mesothelioma and 45 patients with benign asbestos related diseases	Tabata, et al. The diagnostic sensitivity of HMGB1 was 53.8% and the specificity was 97.8%	[26]
	Blood samples of 22 patients with malignant pleural mesothelioma, 20 individuals with long-term exposure to asbestos, 38 patients with benign pleural effusion or malignant pleural effusion caused by non malignant mesothelioma, and 20 healthy controls	HMGB1 serum level can reliably distinguish malignant pleural mesothelioma, asbestos exposed individuals and unexposed control groups. Compared with the healthy control group, the total HMGB1 of malignant pleural mesothelioma and asbestos exposed persons was significantly increased. When the specificity was 100%, the sensitivity was 72.73%, the sensitivity was 100% and the specificity was 5%	[11]

(Continued)Table 1 Protein and metabolite markers

Proteins and metabolites	Origination	Meaning	Reference
Galectin 1	Pleural effusion in 6 cases of mesothelioma, 6 cases of lung adenocarcinoma and 7 cases of benign mesothelioma	Compared with malignant pleural mesothelioma patients, Galectin 1 was highly expressed in lung adenocarcinoma and had been proved to be a good predictor of metastatic cancer and malignant mesothelioma	[27]
Epidermal growth factor receptor (EGFR)	A database of patients treated from 1988 to 2014 from the Department of Thoracic Surgery, Austin Hospital, Melbourne, Australia	Chia, et al. EGFR was overexpressed in 93%(299/321) of patients, and the expression increased by more than half in 64% of cases. Overexpression of EGFR in mesothelioma was more common in epithelioid cell subtypes. EGFR expression was not associated with survival. EGFR conformation associated with EGFR imbalance was found in 8.2% of cases, and the prognosis of these tumor patients was worse	[13]
Syndecan-1 (SDC-1)	42 malignant pleural mesothelioma patients and 40 benign patients	Malignant pleural mesothelioma patients expressed higher levels of SDC-1 than benign patients. It was a more reliable biomarker than MMP, and the related AUCs was 0.93	[12]
Matrix metalloproteinase-7(MMP-7)	49 malignant pleural mesothelioma patients and 307 other tumors	The expression of MMP-7 had high specificity, but only moderate sensitivity. It could be used as a diagnostic basis for differentiating benign and malignant mesothelial cell carcinoma	[15]
Programmed death-1 ligand 1 (PD-L1)	84 mesothelioma	Compared with patients with PD-L1 negative tumors, the expression of PD-L1 positive tumors was higher(30%)(P=0.288) Regardless of the tissue type of malignant pleural mesothelioma, the level of sPD-L1 was significantly higher than that of sPD-1(P=0.001), and there was a positive correlation(P<0.001) The PD-L1 concentration in pleural effusion was correlated with the increasing trend of OS(P=0.062)	[16]
CA125	Serum of 74 patients with malignant peritoneal mesothelioma	The univariate analysis showed that there were significant differences in the survival time of patients with new TNM stage, serum CA125 level, lymph node metastasis and extraperitoneal metastasis (P<0.05). The new TNM staging system and serum CA125 are closely related to the prognosis of malignant peritoneal patients	[28]
Apolipoprotein CI	41 pleural effusion and 48 patients with effusion from other causes	The AUC of apolipoprotein CI was 0.755, and its expression level could be used to identify mesothelioma and other exudates	[29]
Cytokeratin 5 / 6, serum amyloid-2, S100, 14-3-3 epsilon, 14-3-3 theta and fibronectin	Pleural effusion in 5 malignant pleural mesothelioma, 5 cases of lung adenocarcinoma (AC) and 5 cases of breast cancer	Compared with AC patients, the expression of S100 in malignant pleural mesothelioma patients was 2.5 times higher than that in AC patients Compared with AC patients, the expression of cytokeratin 5/6 in malignant pleural mesothelioma patients was greatly increased. Some studies have found that the expression of cytokeratin 5/6 in malignant pleural mesothelioma was 75% to 100%, while the expression in AC was only 2% to 20% The 14-3-3 protein epsilon and 14-3-3 protein theta in malignant pleural mesothelioma exudate were 2.1 and 2.4 times higher than those in AC exudate, respectively Compared with breast cancer samples, the expression of malignant pleural mesothelioma in serum amyloid-2 was significantly up-regulated, which was 2.5 times	[30]
BRCA1 associated protein 1(BAP1)	212 malignant pleural mesothelioma, 12 cases of benign mesothelioma and 42 cases of reactive mesothelioma	BAP1 is often lost in malignant pleural mesothelioma, especially the epithelioid/biphasic subtype, which is usually associated with homozygous BAP1 deletion. BAP1 immunostaining is an excellent biomarker with 100% specificity in the identification of benign and malignant mesothelial hyperplasia	[31]
Vascular epidermal growth factor(VEGF)	108 malignant pleural mesothelioma	The level of serum VEGF in patients with malignant pleural mesothelioma was significantly higher than that in patients with other types of tumors	[8]

Table 2 Potential circulating miRNAs biomarkers of malignant mesothelioma

miRNA	Meaning	Sample	Reference
miR-26b↑ miR-25↑ miR-101↑	Compared with the non cancerous pleural effusion control group, higher levels were detected in the serum of malignant pleural mesothelioma patients Related studies showed that the content of serum was correlated with prognosis	Serum	[34]
miR-126↓	Low levels can distinguish malignant pleural mesothelioma from healthy people and patients with non-small cell lung cancer, which also means poor prognosis Although the accuracy of miR-126 is lower than that of SMRPs, the combination of miR-126 and SMRPs can greatly improve the diagnostic accuracy of malignant mesothelioma In malignant pleural mesothelioma, miR-126 was correlated with SMRPs and VEGF	Serum	[35–36]
miR-223↓ miR-191↓	Lower levels were detected in the serum of patients with malignant pleural mesothelioma compared with the control group of patients with non cancerous pleural effusion	Serum	[34]
miR-20a↓	Lower serum levels were detected in mesothelioma patients compared with healthy controls	Serum	[37]
miR-103/miR-103a-3p↓	The differential diagnosis between mesothelioma patients and those exposed to asbestos: the sensitivity was 83% and the specificity was 71%. The sensitivity and specificity of differential diagnosis between mesothelioma patients and healthy people were 78% and 76%. Combined with mesothelin, the diagnostic performance was improved, with sensitivity of 86% and specificity of 85%	Peripheral blood	[37]
miR-132-3p↓	The sensitivity and specificity of differential diagnosis between mesothelioma patients and asbestos exposed persons were 86% and 61%, respectively. miR-132-3p was combined with mesothelin to improve the diagnostic accuracy	Plasma	[38]
miR-92a↑ miR-29c↑ miR-196b↑	Higher levels were detected in the plasma of mesothelioma patients compared with healthy controls	Plasma	[39]
miR-16↓ miR-17↓ miR-486↓	Lower levels were detected in mesothelioma and asbestos exposed patients compared with healthy controls. The decrease of miR-16 suggested that the prognosis of mesothelioma patients was poor. The anti proliferation effect of miR-486 on mesothelioma cells was centered on the decrease of PIM1 (inhibiting cell proliferation by blocking cells in G_0/G_1 phase)	Plasma/Tissue	[32] [40]
miR-197-3p↑ miR-1281↑ miR-32-3p↑	Higher levels were detected in the serum of patients with malignant pleural mesothelioma compared with healthy controls Bononi, et al. Up regulation of miR-197-3p expression led to the down regulation of FOXO3 gene expression in vivo, and finally blocked autophagy of tumor cells. They speculated that miR-1281 and miR-32-3p also affect oncogenes in vivo in a similar way, resulting in uncontrolled cell proliferation and tumor formation	Serum	[41]
miR-335↑ miR-433↑	Compared with the control group of patients with non cancerous pleural effusion, malignant pleural mesothelioma patients often overexpressed. It suggested poor prognosis	Serum	[34]
miR-625–3p↑	Compared with the healthy control group, higher levels were detected in the plasma of mesothelioma patients, with a diagnostic sensitivity of 73.33% and a specificity of 78.57%	Serum/Plasma	[39]
miR-29c-5p↑	High miR-29c-5p had prognostic value	Plasma/Tissue	[39]
miR-29a↑ miR-516↑	Compared with the control group of patients with non cancerous pleural effusion, higher levels were measured in the serum of patients with malignant pleural mesothelioma	Serum	[34]
miR-145↓ miR-10b↓ miR-320↓	Compared with the healthy control group, lower levels were detected in the tissues of patients with malignant pleural mesothelioma, which had higher sensitivity and specificity in the diagnosis of malignant pleural mesothelioma Pinelli, et al. The decrease of miR-320 was related to the inactivation of p53 pathway and the immune escape of tumor cells caused by PD-L1 overexpression	Serum/Tissue	[42]

Note: Arrows ↑ ↓ indicate that this miRNAs is up / down regulated in mesothelioma patients

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