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多肽组学技术在体液生物标志物中的研究进展

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摘要:多肽组学是蛋白质组学技术的延伸,理论上是指器官、组织、细胞和体液中分子质量小于 10 ku 的全部内源性多肽。体液多肽组包含了丰富的人体病理和生理信息,自 2000 年提出生物标记物研究以来,引起了研究者的广泛关注。本文对多肽组学的研究方法和 3 种体液多肽组生物标志物的发展和应用进行总结,提出多肽组的宽泛性,探讨利用肽段分子质量作为标记物的缺点,指出在多肽组分析的各个步骤都可能引入一定的不确定性。

关键词:质谱;体液;多肽组;生物标记物

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Recent Advances in Peptidomics of Body Fluid Biomarkers

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Abstract: Peptidomics is an extension of proteomics. The target of peptidomicsis endogenous peptides with molecular weight less than 10 ku in organs, tissues, cells and body fluids. Body fluid peptides contain abundant pathological and physiological information of human. The studies of body fluid peptides as biomarkers had attracted tremendous attentions since they were proposed in 2000. This review covered the research methods of peptidomics and the development and application of three kinds of body fluid peptidome biomarkers, which pointed out the versatility of peptidomics. The disadvantages of using peptide molecular weight as biomarkers were also discussed. It indicated that some uncertainty might be introduced in each step of peptide analysis.

Key words: mass spectrometry; body fluids; peptidomics; biomarkers

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分子质量小于 10 ku 的小蛋白质统称为多肽,由内源性肽酶水解前体蛋白质产生,人体的生理或病理变化会动态地反映在蛋白质和肽的产生和代谢上[1-3]。多肽组学(peptidomics)研究体液、细胞、组织等材料中的全部多肽[4],其中,由于体液取样方便,可以为疾病提供可能的生物标志物信息。早期的肽检测主要使用放射免疫分析或酶联免疫吸附试验等免疫分析法,但只能分析已知序列的多肽[5]。近年来,基于质谱的多肽组学技术得以发展,血液、唾液、尿液、汗液、泪液和胸腔积液等体液多肽组学标志物的研究明显增多[2,6-11],尤以前 3 种体液最为常见。多肽组学的分析一般包括样品预处理、内源性肽提取分离、质谱分析、肽鉴定与定量、数据分析等步骤[12],示于图 1。本文将从多肽组学研究方法出发,对多肽的分离、表征、应用和多肽组分析结果的影响因素等方面进行综述。

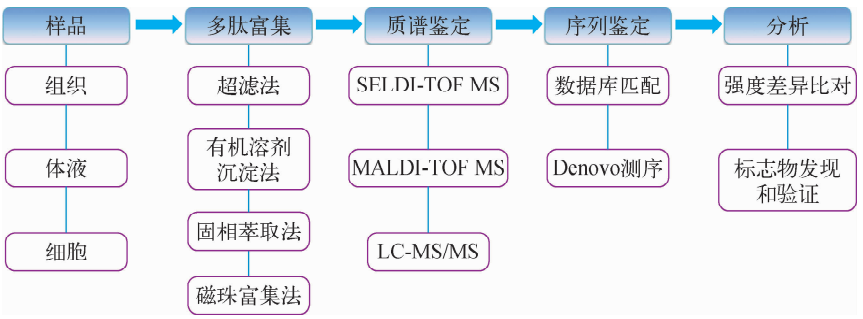

图 1　多肽组生物标志物研究的一般路线

Fig. 1　General route of peptidomics biomarker research

1　多肽的分离方法

为降低高丰度蛋白质、糖、脂质或盐等基底物质的干扰[12],在质谱检测前需进行多肽的分离提取和富集,主要的方法包括有机溶剂沉淀法[13-14]、离心超滤法[14-15]、固相萃取(SPE)法[16-17]和纳米材料磁珠富集法[17-18]等。其中,有机溶剂沉淀法通过加入乙腈等有机溶剂,使溶液中大分子蛋白质沉淀,而肽溶解在有机溶剂中,会形成共沉淀导致肽损失,需要辅助解离试剂进行增溶,提高肽的回收率[19]。Romanova 等[20]使用 2,5-二羟基苯甲酸(DHB)有效地从组织中提取内源性肽,且储存在 DHB 提取介质中的肽提取物可稳定保存多年,该技术简单、重现性好、易于远程制备样品,无需冷冻即可长期保存样品。固相萃取是一种从生物样品中获取内源肽的有效方法,固体吸附剂材料的结构和表面性质在肽的提取中起着重要作用。凝胶过滤色谱(GFC),又称尺寸排阻色谱(SEC),是利用分子大小提取内源性肽的有效方法[12]。Kononikhin 等[21]在使用 LC-MS/MS 法对先兆子痫的尿肽组分析时,采用基于 SPE 和 SEC 2 种不同方案,发现相较于 SPE 法,SEC 法鉴定的肽总数更多,且 SPE 法提取的肽长度不超过 30 个氨基酸。孔祥怡等[17]使用 Zip-Tip C18 固相萃取和氧化石墨烯-磷酸镧纳米复合材料(LaGM)分离唾液多肽组,发现这 2 种方法对多肽的富集有一定的偏好性,所得的肽段分布特征和优势肽段构成存在明显差异,即使用单一分离法只能获得全部多肽组的部分信息。

Ziganshin 等[18]提出一种从丰富的血液蛋白质中解吸低分子质量肽的方法。将稀释血清加热至 98 ℃后保持 15 min,将低分子质量肽从最丰富的血液蛋白质中分离,再使用带有功能化表面的磁珠进行血浆/血清分离,显著增加了 MALDI-TOF MS 检测到的低分子质量肽的数量。Ma 等[22]合成并开发了一种新颖的多功能复合材料 rGO-SnO_2 NRs,该材料包括在还原的氧化石墨烯(rGO)片上垂直排列的介晶 SnO_2 纳米棒(NRs)多功能的亲和探针,结合了 rGO 的疏水性和 SnO_2 的高亲和力 NRs,可通过简单调节洗脱缓冲液来连续富集标准样品和血清样品中的内源肽和磷酸肽,这种二元复合材料表现出对肽富集的高灵敏度和选择性。

Fang 等[23]将固定化金属离子亲和色谱法(IMAC)与介孔材料相结合,提出了铜离子掺杂磁性介孔二氧化硅材料(简称为"磁性介孔材料")Fe_3O_4@mSiO_2-Cu^{2+}对内源性多肽具有显著的敏感性和尺寸专一性。Cheng 等[24]用*RE*PO4(*RE*=La,Nd,Eu)纳米棒修饰的亲和MALDI 板,从复杂的生物样品中选择性捕获和纯化痕量磷酸肽。在基质沉积后,可使用MALDI-TOF MS 直接检测亲和板上的富集磷酸肽,整个过程可在几分钟内完成。近 10 年来,复旦大学、军事医学科学院、大连化物所和长春应化所等多家高校和科研院所在多肽的纳米材料富集方面做了大量的创新性工作,但采用的研究体系通常为牛血清白蛋白等简单体系,如果这些材料能用于体液多肽组等复杂体系,则可以更好地发挥新材料的特性,拓展体液多肽组的研究深度。

2 多肽组的质谱分析方法

基质辅助激光解吸电离飞行时间质谱(MALDI-TOF MS)和电喷雾质谱(ESI-MS)是多肽组分析中最常用的 2 种技术[6,25]。MALDI-TOF 是早期多肽组研究的主要工具,因操作简便、分析速度快、通量高和结果直观而被广泛使用,但由于电离抑制效应,MALDI-TOF 得到的质谱峰数目通常在 100 以内。ESI-MS 常与液相色谱或毛细管电泳联用,获取信息能力随分析时间的增加而增强,所得多肽组序列信息更精准[25-27]。有研究将这 2 种方法结合,先使用 MALDI-TOF MS 等方式分析得到差异肽信息(如质荷比、分子质量)后,再使用 MALDI-TOF/TOF 或 LC-MS/MS 等方式对特定差异肽进行深入分析,从而获得更具体的肽段信息(如肽段序列、肽来源蛋白质等)[13,21,28-38],但对于非特异性酶切的内源性多肽,MALDI-TOF/TOF 的二级质谱断裂效果欠佳。一些研究开发了新型的多肽分离技术,例如使用特殊芯片的免疫印记芯片法(BLOTCHIP-MS),可以将凝胶电泳中分离的肽和蛋白质电转印至免疫印记芯片后直接使用 MALDI-MS 进行质谱分析,省略了染色、提取、装载等中间过程,缩短了分析时间,可以同时分析样本中的游离肽和蛋白质结合肽[29,39-40]。近年来,这 2 种电离方式在生物标志物中的应用情况列于表 1。可以看出,体液多肽组生物标志物在肿瘤、内分泌系统、神经系统、泌尿系统、泌尿生殖系统和呼吸系统等疾病中均具有潜在的应用价值。

表 1 不同质谱方法在疾病的体液生物标志物的应用

Table 1 Application of different mass spectrometry methods in body fluid biomarkers of diseases

分析方法 Analysis method	体液 Body fluid	疾病 Disease	参考文献 Reference
MALDI-TOF MS	脑脊液	阿尔茨海默症	[13]
nLC-MS/MS			
SELDI-TOF MS	脑脊液	烟雾病	[41]
nLC-MS/MS	脑脊液	颅内囊状动脉瘤	[42]
LC-MS/MS	血清	肾透明细胞癌	[14]
nano-LC/ESI-MS/MS	血清	胃腺癌	[43]
Nano LC-LTQ-Orbitrap MS	血清	溃疡性结肠炎	[44]
LC-MS/MS	脐带血	新生儿呼吸窘迫综合征	[45]
LC/MS	胎儿母体血清	法洛四联症	[46]
MALDI-TOF MS	血清	恶性肝肿瘤	[28]
LC-MS/MS			
MALDI-TOF MS	血清	COVID-19	[47]
MALDI-TOF MS	血清	肝细胞癌	[48]
MALDI-TOF MS	血清	类风湿性关节炎	[49]

续表 1

分析方法 Analysis method	体液 Body fluid	疾病 Disease	参考文献 Reference
BLOTCHIP-MS	血清	阿尔茨海默症	[29]
MALDI-TOF/TOF			
LC-MS/MS			
MALDI-TOF MS	血清	急性白血病	[30]
nLC-MS/MS			
MALDI-TOF MS	血清	甲状腺乳头状癌	[31]
nLC-MS/MS			
LC-MS/MS	血清	妊娠期糖尿病	[50]
UHPLC-Q-Orbitrap MS	血清	非小细胞肺癌	[51]
LC-MS/MS	血清	卵巢癌	[52]
LC-MS/MS	血清	儿科 IgA 肾病	[53]
MALDI-TOF MS	血清	结直肠癌	[54]
BLOTCHIP-MS	血清	弥散性血管内凝血	[40]
MALDI-TOF-MS/MS			
CE-MS	尿液	慢性肾病	[55]
MALDI-MS	尿液	急性肾损伤	[32]
CE-MS			
MALDI-MS	尿液	急性肾损伤	[33]
CE-MS			
LC-MS/MS	尿液	慢性肾移植功能障碍	[56]
LC-MS			
HPLC-MS	尿液	1 型糖尿病	[57]
MALDI-TOF MS	尿液	2 型糖尿病	[34]
nLC-MS/MS			
Label-free LC-MS/MS	尿液	肾细胞癌	[58]
MALDI-TOF	尿液	前列腺癌	[35]
LC-MS/MS			
HPLC-MS/MS	尿液	先兆子痫	[21]
CE-MS/MS			
HPLC-MS/MS	尿液	先兆子痫	[59]
CE-MS	尿液	心力衰竭	[60]
MALDI-TOF MS	尿液	重度抑郁症	[36]
nLC-MS/MS			
CE-MS	尿液	系统性红斑狼疮	[61]
MALDI-TOF MS	尿液	坏死性小肠结肠炎	[37]
MALDI-TOF/TOF			
LTQ-Orbitrap MS			
MALDI-TOF MS	唾液	低龄婴幼儿龋	[38]
nLC-MS/MS			
UPLC TQ MS	唾液	口腔鳞状细胞癌	[62]
LC-MS/MS	唾液	口腔鳞状细胞癌	[63]
MALDI-TOF MS	唾液	加速成骨正畸	[64]

3 体液多肽组生物标志物的应用

研究生物标志物的目的是鉴定和区分特定疾病[65]。脑脊液是脑室和中枢神经系统血管周围的无色体液,因此脑脊液的组成会动态地反映中枢神经系统中的许多生理或病理过程[66]。已有大量研究证明,α-突触核蛋白、$A\beta_{42}$、tau、磷酸化 tau 和神经丝蛋白等脑脊液蛋白或多肽可用于诊断阿尔茨海默病[67]、帕金森病[68]和额叶颞叶痴呆[69]等神经退行性疾病。Wijte 等[13]利用 MALDI-TOF 对阿尔茨海默病患者死后脑脊液中的肽进行差异分析,鉴定出游离肽组分中的差异肽来源于 VGF 神经生长因子诱导前体和补体 C4 前体,蛋白结合肽组分中的差异肽来源于 VGF 神经生长因子诱导前体和 α-2-HS-糖蛋白。为了检测脑血管疾病烟雾病的生物标志物,Maruwaka 等[41]通过 SELDI-TOF MS 分析患者脑脊液,发现4 473 u 等 3 个肽段强度显著升高。但是,获得脑脊液样品需要侵入性取样,并且可能引起某些患者的不适或副作用[70],很难对存在的潜在患者进行普遍筛查,需要血液、唾液、尿液等非侵入性和更易获得的生物标志物来源。

3.1 血清和血浆

血液(血清或血浆)是人类所有细胞、组织和器官之间的主要纽带,组织微环境中产生的蛋白水解肽片段可以反映早期病理变化[65],而小尺寸的肽段容易分泌到细胞外间质,从而释放到血液循环中[71]。部分血液肽组学作为疾病潜在生物标志物的相关研究结果列于表 2。相较于脑脊液、尿液等其他体液,血液标志物可诊断的疾病类别更加丰富,涵盖了癌症[14,28,30-31,43-44,48,51-52,54]、肠炎[44]、呼吸病[45,47]、心血管疾病[46]、神经退行性疾病[29]、糖尿病[50]等多种不同类型的疾病[40,49,53]。数据依赖采集是目前多肽组序列鉴定的最常用方法,数据独立采集策略采用的较少,但可能更具优势[14]。

3.2 尿液

尿液是临床诊断最有用的体液之一[61],尿多肽组学可以极大地改善肾脏疾病的诊断与治疗[72]。部分尿液肽组学作为疾病潜在生物标志物的相关研究结果列于表 3。Good 等[55]采用 CE-MS 筛选出 273 种潜在的慢性肾病生物标记物,敏感性和特异性分别为 85.5% 和 100%。Carrick 等[33]通过 MALDI-MS 分析了 95 名败血症患者的尿液样本,采用 CE-MS 鉴定了 39 种尿肽作为伴随败血症的急性肾损伤生物标志物,敏感性和特异性分别为 86% 和 76%,其中部分肽段鉴定为来自胶原链 α-1(Ⅰ)(COL1A1)和 α-1(Ⅱ)(COL1A2)、α-1-抗胰蛋白酶(SERPINA1)、β-2-微球蛋白(B2M)和纤维蛋白原 α 链(FGA)的片段。di Meo 等[58]使用定量无标记 LC-MS 和靶向平行反应监测,鉴定了 9 种在肾癌中显示出明显升高表达的内源肽,证明了非侵入性内源肽作为早期肾癌的潜在诊断和预后标志物的实用性。

除了针对肾相关疾病的研究外,尿肽标志物还应用于其他生殖或泌尿类疾病的诊断,如前列腺癌[35,73-74]、膀胱癌[75]、先兆子痫[21,59]等。M'Koma 等[35]利用 MALDI-TOF 分析了 407 个尿液样品,在 m/z 1 373.1、1 433.5、2 236.3 和 2 484.6 处发现了一系列能够区分前列腺癌和前列腺增生患者的肽段。Kononikhin 等[21]通过 HPLC-MS/MS 检测出包括 SERPINA1 的 C 末端片段(MIEQNTKSPLFMGKVVNPTQK)和白蛋白肽(DAHKSEVAHRFKDLGEENFKALVL)在内的 35 个先兆子痫尿肽标志物。此外,Zhang 等[60]利用 CE-MS 鉴定了 96 种心力衰竭的潜在生物标志物。Wang 等[36]利用 MALDI-TOF MS 确定了 5 个重度抑郁症潜在生物标记物,灵敏度和特异性分别为 91.7% 和 84.6%,并鉴定其中 4 个肽段为血清白蛋白、AMBP 蛋白、HSPG 和载脂蛋白 A-I(APOA1)的片段。

3.3 唾液

Ao 等[38]利用 MALDI-TOF 找到了 3 种可用于鉴定低龄婴幼儿龋的候选唾液肽生物标志物 1 346.6、2 603.5、3 192.8 u。Chi 等[62]在唾液中检测到 5 种口腔鳞状细胞癌生物标志物 MMP1、PADI1、TNC、CSTA 和 MMP3。Neves 等[63]通过 LC-MS/MS 证明,唾液中富含脯氨酸的碱性蛋白 1 的肽片段和蛋白 LCN1、MUC7、PON1、C4BPA、ITIH2、AHSG 可用于鉴别颈淋巴结转移患者。Wu 等[64]采用 MALDI-TOF MS 分析 6 例加速成骨正畸患者的 36 份唾液标本,结果显示有 182 个峰有显著性差异。

表 2　血液肽作为疾病生物标志物的研究与应用

Table 2　Research and application of blood peptides as biomarkers of diseases

疾病 Disease	标志肽段数量 Number of biomarker peptide	部分标志肽段 Part of biomarker peptide	肽段来源蛋白质 Source of peptide	参考文献 Reference
肾透明细胞癌	833	—	—	[14]
胃腺癌	33	—	—	[43]
溃疡性结肠炎	6	ADSGEGDFLAEGGGVR↑	FGA	[44]
		DSGEGDFLAEGGGVR↑	FGA	
		DDPDAPLQPVTPLQLFEGRRN↑	补体 C4A	
		SGEGDFLAEGGGVR↑	FGA	
		EGDFLAEGGGVR↑	FGA	
		GLEEELQFSLGSK↓	补体 C4A	
新生儿呼吸窘迫综合征	251	DLEVLEGGAATL↑	OBSCN	[45]
		ATQDNAHRAEATRRVLERLVLALGPLGPQAVQ↑	CO7A1	
		SGKSKGGK↑	MYH6	
		YLALGLLKLVGTMLG↑	MRP7	
		CLKSVTLSLDGAQT↑	MUC5A	
		KTGLLF↑	LRFN1	
		DECSKDNGGCQHECVNTMGSYMCQCRNGFVLHD↑	E9PD25	
		YVAAKLALGI↑	F8VPD4	
		GPVGGRGPKGDPGSLGPL↑	SCAR3	
		QGPFTTQ↑	PERQ1	
		TQSNLLSVAGRLGLDW↑	PIDD1	
法洛四联症	278	SQGLISAARMVAAAT↓	talin-1	[46]
		IAAATSALVKAASAAQRELVAQGKVGAI↓	talin-1	
COVID-19	25	m/z 6357↓	—	[47]
		m/z 6654↓		
		m/z 6639↓		
		m/z 13886↓		
		m/z 28232↓		
		m/z 7614↑		
		m/z 15123↑		
		m/z 15867↑		
		m/z 28091↑		
肝癌	27	MADEAGSEADHEGTHSTKRGHAKSRPV↓	FGA	[28]
		GHRPLDKKREEAPSLRPAPPPISGGGY↓	FGB	
		NVHSGSTFFKYYLQGAKIPKPEASFSPR↑	ITIH4	
	64	m/z 5247.62↑	—	[48]
		m/z 7637.05↑		
		m/z 1450.87↓		
		m/z 4054.21↓		
		m/z 1073.37↑		
		m/z 3883.64↓		
		m/z 5064.37↑		
		m/z 4644.96↓		
		m/z 5805.51↑		
		m/z 1866.47↓		
		m/z 6579.6↑		

续表 2

疾病 Disease	标志肽段数量 Number of biomarker peptide	部分标志肽段 Part of biomarker peptide	肽段来源蛋白质 Source of peptide	参考文献 Reference
类风湿性关节炎	53	m/z 2367.5↑	—	[49]
		m/z 7767.8↓		
		m/z 1617.46↑		
阿尔茨海默症	4	GHRPLDKKREEAPSLRPAPPPISGGGY↑	FBC	[29]
		TVVQPSVGAAAGPVVPPCPGRIRHFKV↑	AHSG	
		TFPGFFSPMLGEFVSETESRGSESGIFTNTKESSSHHPGIAEFPSRG↑	FAC	
		TLLVFEVQQPFLFVLWDQQHKFPVFMGRVYDPRA↑	PPC1I	
急性白血病	26	SALVETRTIVRFNRPFLMIIVPTDTQNIFFMSKVTNPKQA↑	SERPINA3	[30]
甲状腺乳头状癌	6	m/z 5905.22↓	—	[31]
		SYKMADEAGSEADHEGTHSTKRGHA↓	FGA	
		DSGEGDFLAEGGGVR↓	FGA	
		RNGFKSHALQLNNRQIR↑	补体 C4A/B	
		m/z 6630.37↑	—	
		m/z 1978.22↓	—	
妊娠期糖尿病	297	FSSPNKTG↑	NU214	[50]
		KKLPPAS↑	VIP1	
		RPQGT↑	L1CAM	
		RTYSLTT↑	IRS2	
非小细胞肺癌	201	QGAKIPKPEASFSPR↓	ITIH4	[51]
		CDDYRLC↑	MGP	
卵巢癌	53	VNPFRPGDSEPPPAPGAQRAQMG↓	ZYX	[52]
		NNSNAAEDDLPTVELQGVVPR↓	F13A	
		TNGQLQQPT↓	SNP23	
儿科 IgA 肾病	123	QEKNPLPSKETIEQE↑	—	[53]
		PPPVLAK↓	—	
结直肠癌	24	m/z 1208↑	—	[54]
		m/z 1467↑	—	
		VVSLGSPSGEVSHPR↑	AHSG	
		AILVDLEPGTMDSVR↑	β-tubulin	
		m/z 1656↑	—	
		m/z 4215↓	—	
弥散性血管内凝血	13	TVVQPSVGAAAGPVVPPCPGRIRHFKV↓	AHSG	[40]
		MADEAGSEADHEGTHSTKRGHAKSRPV↓	FGA	
		GHRPLDKKREEAPSLRPAPPPISGGGY↓	FGB	
		HKSEVAHRFKDLGEENFKALVLIAF↓	SA	
		ENGKPGEPGPKGDAGAPGAPGGKGDAGAPGERGPPG↓	COL3A1	
		PGKAGERGVPGPPGAVGPAGKDGEAGAQGPPGPAGPA↓	COL1A1	
		GAEDSLADQAANKWGRSGRDPNHFRPAGLPEKY↑	血清淀粉样 A-2 蛋白	
		SETSRTAFGGRRAVPPNNSNAAEDDLPTVELQGVVPR↓	凝血因子 XIII A 链	

表 3　尿液肽作为疾病生物标志物的研究

Table 3　Research and application of urine peptides as biomarkers of diseases

疾病 Disease	标志肽段数量 Number of biomarker peptide	部分标志肽段 Part of biomarker peptide	肽段来源蛋白质 Source of peptide	参考文献 Reference
慢性肾病	273	—	—	[55]
急性肾损伤	20	—	—	[32]
	39	NGERIEK↓	B2M	[33]
		GppGppGPAGKEG↑	COL1A1	
		IGPpGPAGApGDKG↓	COL1A1	
		SpGPDGKTGPpGPA↓	COL1A1	
		TIDEKGTEAAGAMF↑	SERPINA1	
		DEAGSEADHEGTHSTK↑	FGA	
		TGPAGEpGREGSPGADGPPGRD↑	COL1A2	
慢性肾移植功能障碍	3	m/z 638.03↓	—	[56]
		m/z 645.59↑	—	
		m/z 642.61↑	—	
1 型糖尿病	15	SGSVIDQSRVLNLGPI↑	UMOD	[57]
		SGSVIDQSRVLNLGPITR↓	UMOD	
		SVIDQSRVLNLGPI↑	UMOD	
		SVIDQSRVLNLGPIT↑	UMOD	
		SVIDQSRVLNLGPITR↑	UMOD	
		SVIDQSRVLNLGPITRK↑	UMOD	
		VIDQSRVLNLGPI↑	UMOD	
		DDGGPYGESEAPAPPGPGTRWP↓	LTBP4	
		TGLSMDGGGSPKGDVDPF↓	FXYD2	
		SHTSDSDVPSGVTEVVVKL↑	CLU	
		HTSDSDVPSGVTEVVVKL↑	CLU	
		LSALEEYTKKLNTQ↑	APOA1	
		YGEMADCCAKQEPERNECFLQ↑	ALB	
		VRYTKKVPQVSTPTL↑	ALB	
		TVVQPSVGAAAGPVVPPCPGRIRH↑	AHSG	
2 型糖尿病	2	SSYSKQFTSSTSYNRGDSTFESKSYK↑	FGA	[34]
		CGLRPLFEKKSLEDKTERELLESYIDGRI↑	凝血酶原前体	
肾细胞癌	9	NVINGGSHAGNKLAMQEF↑	—	[58]
		VNVDEVGGEALGRL↑		
		VVAGVANALAHKYH↑		
		SHTSDSDVPSGVTEVVVKL↑		
		IVDNNILFLGKVNRP↑		

续表 3

疾病 Disease	标志肽段数量 Number of biomarker peptide	部分标志肽段 Part of biomarker peptide	肽段来源蛋白质 Source of peptide	参考文献 Reference
前列腺癌	4	m/z 1373.1↑	—	[35]
		m/z 1433.5↑		
		m/z 2236.3↓		
		m/z 2484.6↓		
先兆子痫	35	MIEQNTKSPLFMGKVVNPTQK↑	SERPINA1	[21]
		DAHKSEVAHRFKDLGEENFKALVL↑	ALB	
	7	LRTLNQPDSQLQLTTGNGLF↑	SERPINA1	[59]
		EAIPMSIPPEVKFNKP↑	SERPINA1	
		PMSIPPEVKFNKP↑	SERPINA1	
		LMIEQNTKSPLFMGKVVNPTQK↑	SERPINA1	
		MIEQNTKSPLFMGKVVNPTQK↑	SERPINA1	
		IEQNTKSPLFMGKVVNPTQK↑	SERPINA1	
		EQNTKSPLFMGKVVNPTQK↑	SERPINA1	
心力衰竭	96	—	—	[60]
重度抑郁症	29	TGSPAPTIHWSKLRSPLPWQHRLEGDTLII PRVAQQDSGQY↑	HSPG	[36]
		SALEEYTKKLNTQ↑	APOA1	
		m/z 8307.22↓	—	
		YVPKEFNAET↑	ALB	
		LTKKFSRHHGPTITAKLYGRAPQLRETL↓	AMBP	
系统性红斑狼疮	65	—	WRNIP1	[61]
			COL1A1	
			COL3A1	
			COL4A1	
			COL5A1	
			COL26A1	
			COL1A2	
			FOXO1	
			Mucin-12	
			UMOD	
坏死性小肠结肠炎	36	DEAGSEADHEGTHSTKR↑	FGA	[37]
		DEAGSEADHEGTHSTKRG↑	FGA	
		DEAGSEADHEGTHSTKRGHAKSRPV↑	FGA	

注:p 表示羟脯氨酸

综上,多肽组生物标志物是一个较宽泛的概念,肽段分子质量、肽段序列及其归属的蛋白质都可用于表征。因此,标志物的数量差异较大,并且肽段强度的上调和下调都与疾病状态相关。值得注意的是,尽管以肽段分子质量为多肽组标记物的指标被广泛使用,MALDL-TOF 谱图中的 1 个质谱峰也可能代表多个肽段。例如,血清中 Apolipoprotein A-Ⅱ的肽段 YFVELGTQPATQ 分子质量为1 352.668 u,而归属于 Prothrombin 的肽段 FEKKSLEDKTE 分子质量为 1 352.684 u,Hemoglobin subunit alpha 的降解肽段 QLSELHCDKLHVD(Gln->pyro-Glu@ N-term; Trioxidation(C)@ 7)和 SLDKFLASVSTVLTS 分子质量分别为1 566.704、1 566.861 u,而同一样本中 Profilin-1 的降解肽段 NITPAEVGVLVGKDR 分子质量为1 566.874 u,飞行时间质谱分辨率不足以区分这些肽段,在尿液和唾液中也有类似现象。因此,用分子质量(质荷比)表征多肽组得到的信息量不仅少,而且不够精准。

4　多肽组分析结果的不确定性

虽然研究多肽组生物标记物的报道较多,但是真正用于临床的却较少,原因之一可能是对多肽组分析结果不确定性的认识尚不够充分。早期研究表明[76],尿液收集方式与收集时间等外源性变量因素和尿液 pH 值、盐与蛋白质的浓度以及血液与细菌干扰等内源性变量因素,均会显著影响肽谱分析的结果。唾液成分也会受到收集方法和唾液流动刺激程度的影响,并且唾液含有来自宿主和口腔微生物的蛋白水解酶,这些酶会影响某些标志物的稳定性,并在收集唾液样本后持续降解,导致肽谱的巨大变化,从而限制了肽组学分析的可重复性[77]。吴杰等[78]采用 MALDI-TOF/TOF 技术研究尿液标本的收集储存方法、冻存条件、反复冻融次数等多个实验流程中的影响因素,发现不同性别组间尿液多肽谱未见明显差异,且 5 次以内有限冻融对出峰没有影响。本课题组[79]考察了将唾液样品分别置于 -80 ℃、-20 ℃冻存 6 个月后对唾液多肽组的影响,发现-20 ℃冻存不仅会导致样品中低含量肽段的减少和消失,高含量肽段也会发生一系列的进一步降解。即使是对于完全相同的样本,纳升液相色谱-质谱分析法的重现性对实验结果也有影响,我们利用纳升液相色谱-高分辨质谱对健康人的尿液多肽组进行 7 次平行分析,发现多肽组的单次分析结果具有一定的随机性和相对的稳定性。增加平行实验次数会扩大多肽组数据集,但测定 3 次以上后增加幅度减小。相比于肽段,利用降解蛋白质为多肽组的生物标志物更稳健[80]。目前,大多数多肽组学研究只用一种搜库软件解析串联质谱数据,我们使用 Peaks studio 8.5(PS)和 Protein Pilot Software4.0(PP)两种软件分析了 10 个随机唾液样本的多肽组数据,发现多肽组的分析结果与搜库方法相关,某些肽段只能被 PS 或 PP 一种软件鉴定,两种软件具有互补性[81]。因此,在样本的取样、保存、多肽提取富集、液相色谱-质谱分析和数据库搜索的各环节都可能产生分析结果的变化,质谱灵敏度的增加和肽段分析能力的提高可以更清晰地观察这些变化。

5　总结与展望

体液多肽组标志物具有广泛的临床需求,血液标志物应用广泛,尿液多肽组标志物多应用于肾病相关疾病,唾液多肽组则较多用于口腔疾病,但是,尿液和唾液中也含有与血液相同或相似的多肽和降解蛋白质,同样参与生理循环,因此在其他疾病中也具有更广阔的应用前景。值得注意的是,目前对健康人体液多肽组生理波动的研究很少,对它们的本质特征和分布规律缺少深层次的认识。为使多肽组学标记物真正应用于临床,需规范和优化多肽组分析方法,降低分析结果的不确定性。加强对各种不同体液多肽组本质特征和规律的探索,区分多肽组结果中的生理变化和病理变化,研究体液多肽组在健康人体内的基本组成和波动幅度。

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