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神秘果干预肿瘤免疫的潜在机制研究

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摘　要: 目的：研究神秘果干预肿瘤免疫药效学物质基础依据以及可能机制。方法：利用液相-质谱分析明确神秘果的成分，之后利用网络药理学以及生物信息学方法系统研究了神秘果作用于肿瘤免疫的作用靶点,同时明确神秘果药食同源的可能性。结果：液相-质谱实验得到神秘果的可能含有成分 360 种，与肿瘤免疫数据库对比后得到可干预肿瘤免疫相关成分 42 个，干预相关靶点 55 个，筛选后得到靶点网络中关键靶点为 TSHR、TP53、MAPK1、HIF1A、CA9。进一步收集文献中实验信息及分子对接实验验证神秘果的不同成分可影响选取靶点进而干预肿瘤免疫。结论：通过以上实验发现神秘果成分与肿瘤免疫相关靶点可能具有相互作用，为神秘果直接作用肿瘤研究铺垫基础。

关键词: 神秘果, 肿瘤, 肿瘤免疫, 网络药理学, 液相-质谱

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本文网刊:

Study on the Potential Mechanism of *Synsepalum dulcificum* Interfering with Tumor Immunity

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Abstract: Objective: To study the material basis and possible mechanism of the pharmacodynamics of *Synsepalum dulcificum*'s intervention in tumor immunity. Methods: The study used liquid phase-mass spectrometry analysis to clarify the composition of *Synsepalum dulcificum*, and then used the network pharmacology and bioinformatics methods to systematically study the specific impact of *Synsepalum dulcificum* on tumor immunity. At the same time, it was clear that *Synsepalum dulcificum* medicine and food were homologous.Results: The liquid-mass spectrometry experiment revealed that *Synsepalum dulcificum* may contain 360 components. After comparing with the tumor immunity database, 42 components related to tumor immunity and 55 related targets were obtained. After screening, key targets in the target network were obtained. These were TSHR, TP53, MAPK1, HIF1A, CA9. Further collection of experimental information in the literature and molecular docking experiments verified that the different components of *Synsepalum dulcificum* could affect the selection of targets and interfere with tumor immunity. Conclusion: Through the above experiments, it is found that the components of *Synsepalum dulcificum* may interact with tumor immune-related targets, paving the way for the research on the direct effect of *Synsepalum dulcificum* on tumors.

Key words: *Synsepalum dulcificum*; tumor; tumor immunity; network pharmacology; liquid phase-mass spectrometry

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癌症是全球第二大死亡原因[1]。研究认为癌症的起始与发展不仅与遗传改变相关。不合理饮食、久坐的生活方式和异常肥胖也被认为是肿瘤发生危险因素[2]。目前，尚未发现癌症的明确成因以及显著有效治疗手段，一线治疗方法仍为手术及放射性疗法，但对人体有严重副作用，比如身体衰弱、免疫功能下降及神经毒性等副作用。机体免疫功能与肿瘤发生发展密切相关，人体可以通过不同的免疫途径启动抗肿瘤免疫应答，从而达到抗肿瘤功能。肿瘤免疫治疗相比其他治疗方式具有治疗范围广，临床预后好，可适用于各种癌症类型等优点。某些天然活性物质有提高免疫功能，具有预防肿瘤的发生，减少转移和复发等特点[3]。因此探究具有抗肿瘤活性且低毒副作用的天然活性物质迫在眉睫。

神秘果（*Synsepalum dulcificum*）是热带西非的土产水果，因果肉可以将酸味转变为甜味的特点被称神秘果。神秘果因其成分丰富具有巨大潜在保健价值，目前研究发现其果实、种子及叶等部位的提取物均有不同药理活性，比如降血脂、抗尿酸、抑菌、抗惊厥、抗疲劳等活性[4]。具有诸多生物活性的原因是其果肉中富含酚类化合物（15.8%）和类黄酮（11.9%），果皮含有更高含量的酚类化合物（36.7%）和类黄酮（51.9%）[5]。一般认为酚类和类黄酮含量与抗氧化活性及抗肿瘤之间存在密切关系，高含量的酚类及类黄酮使得神秘果具有干预肿瘤的重要潜在能力[6]。已经报道神秘果提取物可以在体外减少某些恶性肿瘤的增殖，如减少人黑色素瘤、结直肠癌细胞系的增殖和抗人慢性髓系白血病细胞（K562）活性[7−8]。即使神秘果可以干预肿瘤发生发展，但是对于这种果实的抗肿瘤的机制研究仍鲜有报道。

本研究通过液相-质谱联用技术及网络药理学研究手段探究神秘果在肿瘤免疫方面可能发挥的作用，为神秘果的开发利用铺垫基础，同样为抗肿瘤食药开发提供参考。

1　材料与方法

1.1　材料及仪器

神秘果　购自广东江门，经内蒙古医科大学研究员鉴定为山榄科神秘果属植物，−80 ℃ 保存；甲醇、乙腈、甲酸　色谱纯；其他试剂　均为分析纯，天津大茂化学试剂厂。

Triple TOF 5600/6600 质谱仪　美国 AB SCIEX；Agilent 1290 Infinity LC 超高压液相色谱仪　美国 Agilent；Eppendorf5430R 低温高速离心机　德国 Eppendorf；色谱柱：ACQUITY UPLC BEH Amide (1.7 μm, 2.1 mm×100 mm column)、ACQUITY UPLC HSS T3 (1.8 μm, 2.1 mm×100 mm column)　美国 Waters。

1.2　实验方法

1.2.1　液相色谱-质谱方法获得成分

1.2.1.1　样品预处理　取神秘果样品适量，去核，冻干后成粉供精密称取，−80 ℃ 保存。

上机样本制备：−80 ℃ 取出样本，称量 80 mg，加入 200 μL 水匀浆，涡旋 60 s，加入 800 μL 甲醇乙腈溶液（1∶1, v/v），涡旋 60 s，低温超声 30 min，2 次，−20 ℃ 放置 1 h 沉淀蛋白，14000 ×g，4 ℃ 离心 20 min，取上清冷冻干燥，−80 ℃ 保存，上机样品平行两次实验。

1.2.1.2　液相色谱-质谱分析　液相色谱条件：样品采用 Agilent 1290 Infinity LC 超高效液相色谱系统 (UHPLC)HILIC 色谱柱进行分离；柱温 25 ℃；流速 0.3 mL/min；流动相组成 A：水+25 mol/L 乙酸铵+25 mmol/L 氨水，B：乙腈；梯度洗脱程序如下：0~0.5 min, 95% B；0.5~7 min, B 从 95% 线性变化至 65%；7~8 min, B 从 65% 线性变化至 40%；8~9 min, B 维持在 40%；9~9.1 min, B 从 40% 线性变化至 95%；9.1~12 min, B 维持在 95%；整个分析过程中样品置于 4 ℃ 自动进样器中。为避免仪器检测信号波动而造成的影响，采用随机顺序进行样本的连续分析。

质谱条件：分别采用电喷雾电离 (ESI) 正离子和负离子模式进行检测。样品经 UHPLC 分离后用 Agilent 6550 质谱仪进行质谱分析。ESI 源条件如下：气体温度：250 ℃，干燥气体：16 L/min，雾化气压力：20 Psig，壳气温度：400 ℃，壳气流量：12 L/min，Vcap：3000 V，喷嘴电压：0 V，Fragment：175 V，质量范围：50~1200，采集速率：4 Hz，循环时间：250 ms。样本检测完毕后，采用 AB Triple TOF 6600 质谱仪对代谢物进行鉴定，采集样品的一级、二级谱图。ESI 源条件如下：辅助气压 1(Gas1)：40，辅助气压 2(Gas2)：80，气帘气体：30，离子源温度：650 ℃，喷雾电压±5000 V(正负两种模式)；二级质谱采用信息关联扫描获得，并且采用高灵敏度模式，定性离子对去簇电压：±60 V(正负两种模式)，碰撞能量：(35±15)eV，IDA 设置：不包括同位素 4 Da，周期监测的候选离子：10。数据采集是按质量范围进行分段：50~300、290~600、590~900、890~1200，从而扩大二级谱图的采集率，每个方法每段采集四个重复。所采集获得的数据，分别使用 MetDDA 和 LipDDA 方法，进行代谢物的结构鉴定。

1.3　神秘果干预肿瘤免疫相关成分

为明确神秘果影响肿瘤免疫的物质基础，首先将液相-质谱技术获得的化合物在 PubChem Compound (https://www.ncbi.nlm.nih.gov/pccompound) 中搜索转化对应唯一标识符 Smiles，借助中药肿瘤免疫数据库 TCMIO[9] (http://tcmio.xielab.net/) 寻找神秘果成分中含有干预肿瘤免疫相关化合物，明确具体免疫相关化学成分及收录对应靶点。

1.4　靶点生物学功能信息

为更好地了解神秘果中相关免疫化合物靶点的生物学功能，将整理得到的化合物靶点信息利用 R 语言包 clusterProfiler[10] 富集分析，经过合理统计

学阈值明确对应的生物学功能，包括生物过程(biologicalprocess, BP)、分子功能(molecular function, MF)和细胞成分(cellular component, CC)。

1.5 化合物-靶点关系分析

根据成分与相关靶点的关系可构建神秘果作用肿瘤免疫的生物关系网络拓扑图，通常在生物关系网络中，居于关键位置的基因起到的调控的作用更加重要，借助 Cytoscape 软件构建化合物-靶点互作网络，并从网络中选取核心靶点进一步分析。

1.6 核心靶点在不同肿瘤的表达差异

挑选出调控网络的关键基因后，为明确目标靶点在不同肿瘤中的表达差异，借助 GEPIA 数据库[11](http://gepia.cancer-pku.cn/) 记录的 33 种肿瘤中与正常样本数据进行对比，统计条件为表达值经过对数处理，差异统计方法采用方差分析，|Log2FC|>1，P<0.01，对比数据包括 TCGA 及 GTEx 数据。

1.7 化合物影响通路

进一步了解神秘果影响肿瘤免疫的信号通路，通过 CTD 数据库[12](http://ctdbase.org/)富集相关化合物的信号通路，以 P<0.01 进行筛选。

1.8 神秘果中成分与靶点的相互作用

前面工作得到在肿瘤中有明显表达差异的基因，为明确神秘果相关成分对得到的靶点具体相互作用，通过文献及分子对接技术进行验证。神秘果含有化合物可能直接参与靶点基因的表达过程以及产物的翻译修饰途径，还可能与蛋白质直接相互作用进而影响蛋白质功能，借助 TCMSP 数据库收录的化学物质结构信息，下载神秘果相关化学成分结构并保存为 MOL2 格式，使用 Discovery Studio(DS)软件进行化学成分配体的处理。使用 RCSB 数据库(http://www1.rcsb.org/)下载蛋白质结构文件。用 DS 软件进行蛋白预处理，包括清除原配体，去水加氢，施加力场等，LibDock 执行分子对接计算并分析所得结果。

2 结果与分析

2.1 神秘果肉成分的鉴定

平行两组样品经 UHPLC-Q-TOF MS 分析后得到的典型 TIC 图谱,如图 1 所示。通过保留时间(tR)，裂解规律以及数据库的搜索得到样品共有成分为 181 种，数据未给出。

2.2 神秘果果肉干预肿瘤免疫相关成分信息

经过搜索对比，在 TCMIO 数据库中得到神秘果可干预肿瘤免疫相关成分 42 个，同时整理其干预相关靶点 55 个。化合物详细信息表 1。

2.3 神秘果影响肿瘤免疫靶点生物过程、分子功能、细胞成分结果

整理分析得到神秘果影响肿瘤免疫相关靶点 55 个，经过 GO 注释分析及 KEGG 通路分析，筛选得到 P 值及 FDR 值均小于 0.01，如图 2 所示，各项

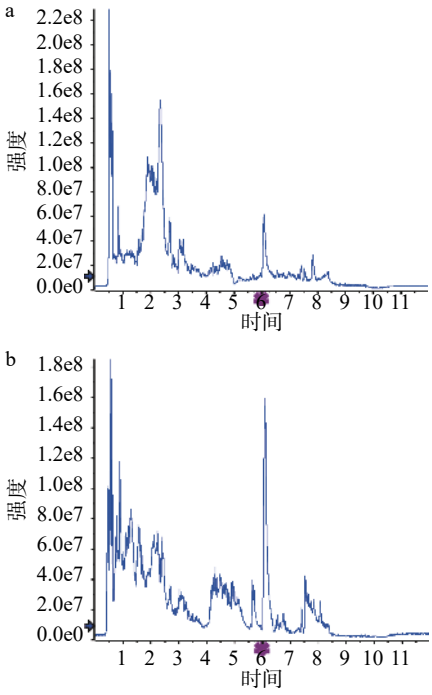


图 1 神秘果离子模式 TIC 图谱

Fig.1 TIC spectrum of *Synsepalum dulcificum* ion mode

注: a: 样品的正离子模式; b: 样品的负离子模式。

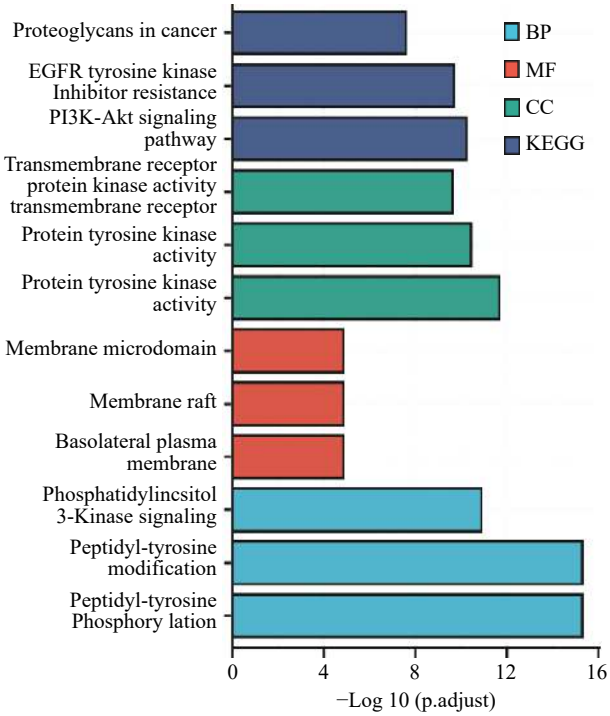


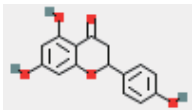
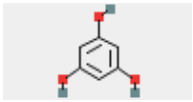
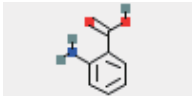
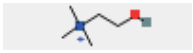
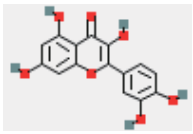
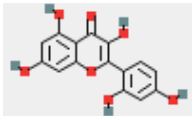
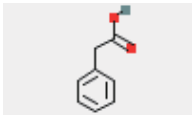
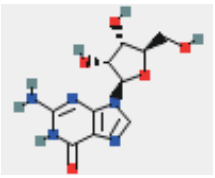
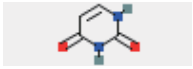
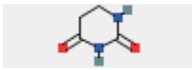
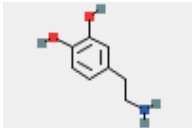
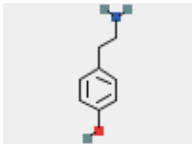
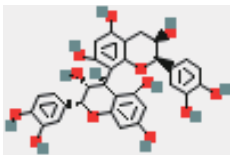
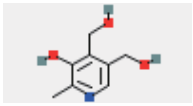
图 2 神秘果干预肿瘤免疫相关靶点的 GO 功能富集及 KEGG 通路分析

Fig.2 GO function enrichment and KEGG pathway analysis of *Synsepalum dulcificum* intervention on tumor immune-related targets

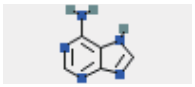
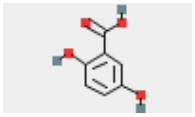
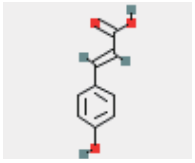
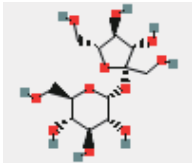
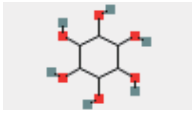
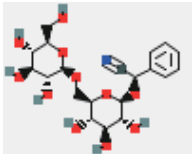
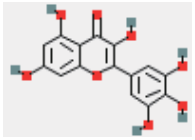
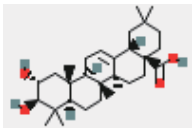
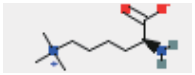
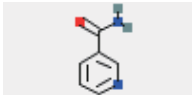
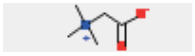
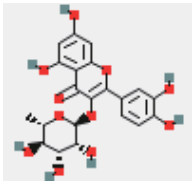
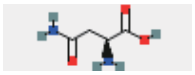
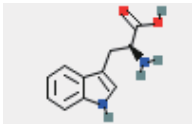
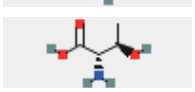
P 值前三信息,主要结果生物过程为肽酰酪氨酸磷酸化、肽酰酪氨酸修饰、磷脂酰肌醇 3-激酶信号，分子功能为蛋白酪氨酸激酶活性、跨膜受体蛋白酪氨酸激酶活性、跨膜受体蛋白激酶活性，细胞定位为基底外侧质膜、膜筏、膜微区，KEGG 为 PI3K-AKT 信号

表 1　神秘果干预肿瘤免疫相关成分信息

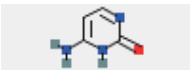
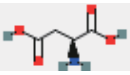
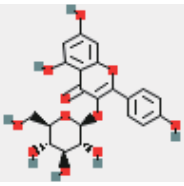
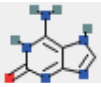
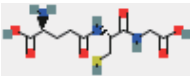
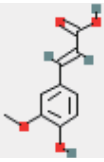
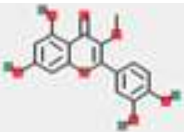
Table 1　Information on relevant components of *Synsepalum dulcificum* 's intervention in tumor immunity

序号	化合物	结构	Smiles	Inchikey	CAS
1	Naringenin		[C@H]1(CC(=O)c2c(cc(cc2O1)O)O)c1ccc(cc1)O	FTVWIRXFELQLPI-ZDUSSCGKSA-N	480-41-1
2	1,3,5-Benzenetriol		c1(cc(cc(c1)O)O)O	QCDYQQDYXPDABM-UHFFFAOYSA-N	108-73-6
3	Anthranilic acid (Vitamin L1)		c1(c(cccc1)N)C(=O)O	RWZYAGGXGHYGMB-UHFFFAOYSA-N	118-92-3
4	Choline		C(CO)[N](C)(C)C	GDPPXFUBIJJIKR-UHFFFAOYSA-N	62-49-7
5	Quercetin		c1(cc(c2c(c1)oc(c(c2=O)O)c1ccc(c(c1)O)O)O)O	REFJWTPEDVJJIY-UHFFFAOYSA-N	117-39-5
6	Morin		c12c(oc(c(c1=O)O)c1c(cc(cc1)O)O)cc(cc2O)O	YXOLAZRVSSWPPT-UHFFFAOYSA-N	480-16-0
7	Phenylacetic acid		c1cccc(c1)CC(=O)O	WLJVXDMOQOGPHL-UHFFFAOYSA-N	103-82-2
8	Palmitic acid		C(=O)(CCCCCCCCCCCCCCC)O	IPCSVZSSVZVIGE-UHFFFAOYSA-N	57-10-3
9	Stearic acid		C(CCCCCCCCCCC)CCCCCC(=O)O	QIQXTHQIDYTFRH-UHFFFAOYSA-N	57-11-4
10	Guanosine		c12n([C@H]3O[C@@H]([C@H]([C@H]3O)O)CO)cnc1c(=O)[nH]c(n2)N	NYHBQMYGNKIUIF-BDXYJKHTSA-N	118-00-3
11	Uracil		[nH]1c(=O)[nH]c(=O)cc1	ISAKRJDGNUQOIC-UHFFFAOYSA-N	66-22-8
12	Dihydrouracil		N1C(=O)CCNC1=O	OIVLITBTBDPEFK-UHFFFAOYSA-N	504-07-4
13	Dopamine		Oc1cc(CCN)ccc1O	VYFYYTLLBUKUHU-UHFFFAOYSA-N	51-61-6
14	Heptadecanoic acid		OC(=O)CCCCCCCCCCCCCCCC	KEMQGTRYUADPNZ-UHFFFAOYSA-N	506-12-7
15	Tyramine		Oc1ccc(CCN)cc1	DZGWFCGJZKJUFP-UHFFFAOYSA-N	51-67-2
16	Procyanidin B2		c1(cc(c2c(c1)O[C@@H]([C@@H]([C@H]2c1cc(cc(c2c1O[C@@H]([C@@H](C2)O)c1cc(c(cc1)O)O)O)O)O)c1cc(c(cc1)O)O)O)O	XFZJEEAOWLFHDH-NFJBMHMQSA-N	29106-49-8
17	Pyridoxine		Oc1c(c(CO)cnc1C)CO	LXNHXLLTXMVWPM-UHFFFAOYSA-N	65-23-6

续表 1

序号	化合物	结构	Smiles	Inchikey	CAS
18	Adenine		[nH]1cnc2c(N)ncnc12	GFFGJBXGBJISGV-UHFFFAOYSA-N	73-24-5
19	Gentisic acid		c1(c(cc(cc1)O)C(=O)O)O	WXTMDXOMEHJXQO-UHFFFAOYSA-N	490-79-9
20	4-Hydroxycinnamic acid		C(=O)(/C=C/c1ccc(cc1)O)O	NGSWKAQJJWESNS-ZZXKWVIFSA-N	501-98-4
21	Sucrose		OC[C@H]1O[C@H](O[C@@]2(CO)[C@@H](O)[C@H](O)[C@@H](CO)O2)[C@H](O)[C@@H](O)[C@@H]1O	CZMRCDWAGMRECN-UGDNZRGBSA-N	57-50-1
22	myo-Inositol		C1(C(C(C(C(C1O)O)O)O)O)O	CDAISMWEOUEBRE-UHFFFAOYSA-N	87-89-8
23	Myristic acid		C(=O)(CCCCCCCCCCCCC)O	TUNFSRHWOTWDNC-UHFFFAOYSA-N	544-63-8
24	Amygdalin		c1(ccccc1)[C@H](C#N)O[C@H]1[C@H](O)[C@@H](O)[C@H](O)[C@H](O1)CO[C@H]1[C@H](O)[C@@H](O)[C@H](O)[C@H](O1)CO	XUCIJNAGGSZNQT-JHSLDZJXSA-N	29883-15-6
25	Myricetin		c1(c(cc(cc1O)c1c(O)c(=O)c2c(o1)cc(cc2O)O)O)O	IKMDFBPHZNJCSN-UHFFFAOYSA-N	529-44-2
26	Maslinic Acid		[C@@]12([C@]3(C(=CC[C@@H]1[C@@]1([C@@H](CC2)C([C@H]([C@@H](C1)O)O)(C)C)C)[C@H]1[C@@](CC3)(CCC(C1)(C)C)C(=O)O)C)C	MDZKJHQSJHYOHJ-LLICELPBSA-N	4373-41-5
27	N6,N6,N6-Trimethyl-L-lysine		C[N+](C)(C)CCCC[C@@H](C(=O)[O-])N	MXNRLFUSFKVQSK-QMMMGPOBSA-N	19253-88-4
28	Nicotinamide		c1(cccnc1)C(=O)N	DFPAKSUCGFBDDF-UHFFFAOYSA-N	98-92-0
29	Betaine		OC(=O)C[N](C)(C)C	GMTCLSZGPOBNLA-UHFFFAOYSA-N	107-43-7
30	Quercitrin		c1(c(c(=O)c2c(o1)cc(cc2O)O)O[C@H]1[C@@H]([C@@H]([C@H]([C@@H](O1)C)O)O)O)c1cc(c(cc1)O)O	OXGUCUVFOIWWQJ-HQBVPOQASA-N	522-12-3
31	L-Asparagine		C(=O)([C@@H](N)CC(=O)N)O	DCXYFEDJOCDNAF-REOHCLBHSA-N	32640-57-6
32	L-Tryptophan		c12c(C[C@@H](C(=O)O)N)c[nH]c1cccc2	QIVBCDIJIAJPQS-VIFPVBQESA-N	73-22-3
33	L-Threonine		O[C@@H]([C@H](N)C(=O)O)C	AYFVYJQAPQTCCC-GBXIJSLDSA-N	72-19-5

续表 1

序号	化合物	结构	Smiles	Inchikey	CAS
34	Cytosine		n1c(cc[nH]c1=O)N	OPTASPLRGRRNAP-UHFFFAOYSA-N	71-30-7
35	L-Aspartate		C(=O)([C@@H](N)CC(=O)O)O	CKLJMWTZIZZHCS-REOHCLBHSA-N	56-84-8
36	Indole		[nH]1c2c(cc1)cccc2	SIKJAQJRHWYJAI-UHFFFAOYSA-N	120-72-9
37	Astragalin		c12c(oc(c(c1=O)O[C@@H]1O[C@@H]([C@H]([C@@H]([C@H]1O)O)O)CO)c1ccc(cc1)O)cc(cc2O)O	JPUKWEQWGBDDQB-QSOFNFLRSA-N	480-10-4
38	2-Hydroxyadenine		c12c(c(nc(n1)O)N)[nH]cn2	DRAVOWXCEBXPTN-UHFFFAOYSA-N	3373-53-3
39	Glutathione		C(CC(=O)N[C@@H](CS)C(=O)NCC(=O)O)[C@@H](C(=O)O)N	RWSXRVCMGQZWBV-WDSKDSINSA-N	70-18-8
40	trans-Ferulic acid		c1(cc(c(cc1)O)OC)/C=C/C(=O)O	KSEBMYQBYZTDHS-HWKANZROSA-N	1135-24-6
41	Quercetin 3'-methyl ether		c1(c(OC)c(=O)c2c(O)cc(O)cc2o1)c1cc(O)c(O)cc1	WEPBGSIAWZTEJR-UHFFFAOYSA-N	1486-70-0
42	4-Guanidinobutyric acid		C(=O)(CCCNC(=N)N)O	TUHVEAJXIMEOSA-UHFFFAOYSA-N	463-00-3

通路、EGFR 酪氨酸激酶抑制剂耐药、肿瘤中蛋白聚糖相关途径。

2.4　筛选核心化合物及靶点

将得到的化合物及对应靶点相互关系导入 Ctyoscape 中，接借助 Networkanalyzer 计算网络拓扑参数，Degree 值前五的基因为核心靶点分别为：*TSHR*(Degree=42)、*TP53*(Degree=39)、*MAPK1*(Degree=39)、*HIF1A*(Degree=30)、*CA9*(Degree=28)继续研究，见图 3。

2.5　核心靶点的临床意义

筛选得到核心靶点，也被认为神秘果影响肿瘤免疫的主要作用靶点，利用 Gepia 内置数据得到核心基因在不同肿瘤中差异情况不一，见图 4~图 8。*CA9* 在 ACC、BLCA、CESC、COAD、ESCA、GBM、HNSC、KIRC、LUAD、LUSC、OV、PAAD、READ、UCEC、UCS 中高表达，而在 LAML、SKCM、STAD、TGCT 中低表达，*HIF1A* 在 ESCA、GBM、HNSC、LGG、PAAD、STAD 高表达，*MAPK1* 在 LGG、PAAD、STAD 中高表达，在

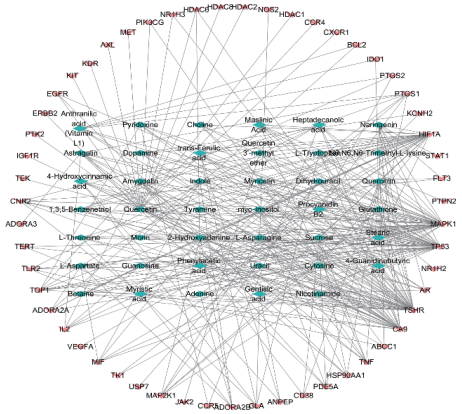


图 3　神秘果干预肿瘤免疫的“成分-靶点”网络

Fig.3　"Component-target" network of *Synsepalum dulcificum* intervening tumor immunity

注：菱形图标为神秘果影响肿瘤免疫成分，三角图标为相关成分影响基因。

ACC、DLBC、LAML 低表达，*TP53* 在 COAD、DLBC、GBM、LAML、LGG、LUSC、OV、PAAD、READ、STAD、TGCT、THYM、UCEC 均高表达，

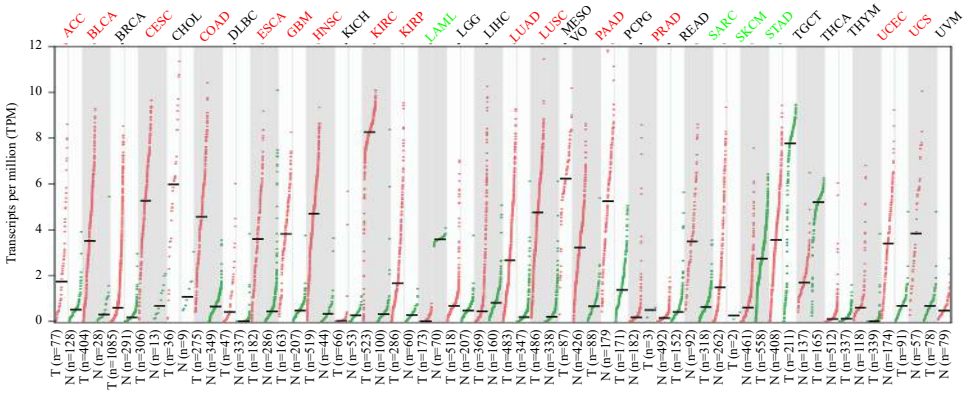


图 4　*CA9* 在不同肿瘤中表达差异

Fig.4　Differential expression of *CA9* gene in different tumors

注: 图中上横坐标为肿瘤类型, 下横坐标为肿瘤对应患者及对照样本; 图 5~图 8 同。

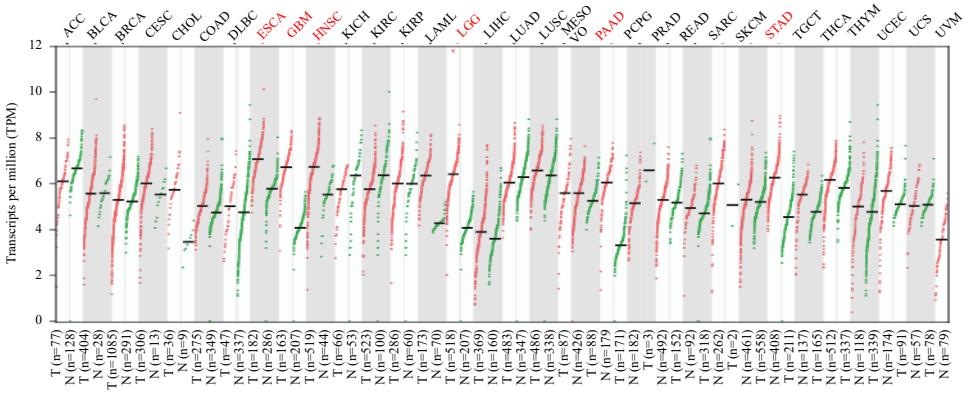


图 5　*HIF1A* 在不同肿瘤中表达差异

Fig.5　Differential expression of *HIF1A* gene in different tumors

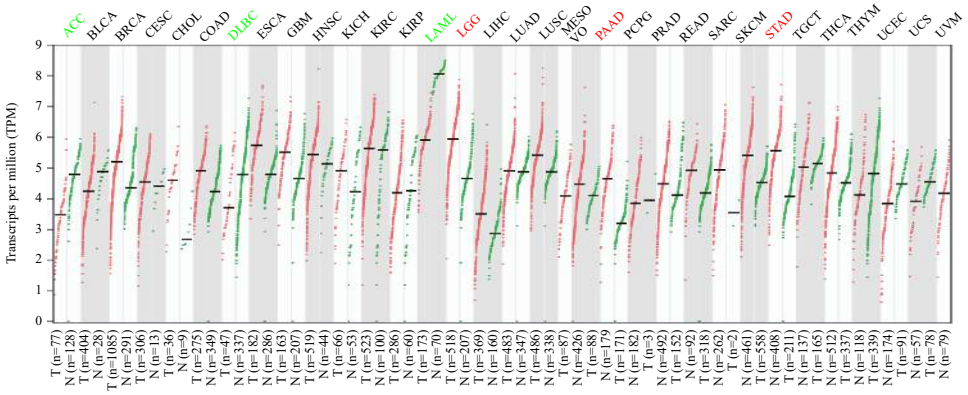


图 6　*MAPK1* 在不同肿瘤中表达差异

Fig.6　Differential expression of *MAPK1* gene in different tumors

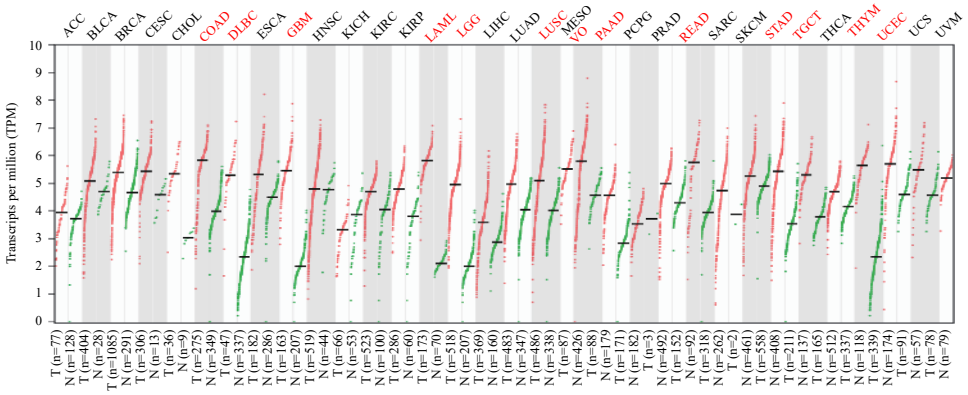


图 7　*TP53* 在不同肿瘤中表达差异

Fig.7　Differential expression of *TP53* gene in different tumors

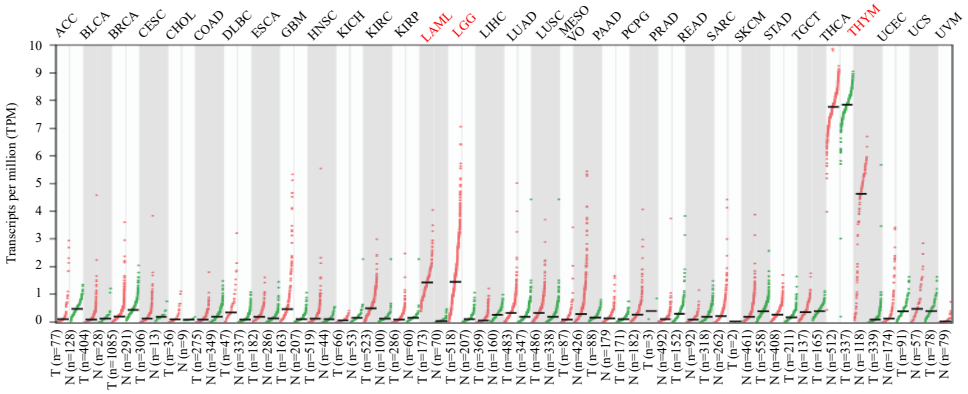


图 8　*TSHR* 在不同肿瘤中表达差异

Fig.8　Differential expression of *TP53* gene in different tumors

TSHR 在 LAML、LGG、THYM 高表达。发现即使同一靶点在不同肿瘤的表达趋势也不一致，证明肿瘤免疫的复杂性和多面性。

2.6　相关化合物影响的信号通路

明确神秘果相关化合物影响体内的信号通路，筛选后结果得到 1479 条信号通路，着重分析 *P* 值排名前十信号通路，可见神秘果相关成分影响的信号通路为免疫系统、先天免疫系统等途径(表 2)。

2.7　化合物对核心基因的影响

通过文献检索，对于上文筛选的神秘果影响肿瘤免疫的 42 种化合物以及筛选的关键化合物进行搜索，得到化合物与核心基因的相互关系。可见神秘果众多化合物可影响多个靶点，佐证文献见表 3。

2.8　分子对接结果

筛选得到核心蛋白 TSHR(PDB：2xwt)、TP53(PDB：3q01)、MAPK1(PDB：4fv6)、HIF1A(PDB：1l8c)、CA9(PDB：6tl5)立体结构与 42 种化合物进行分子对接，仅展示对接分数第一的化合物与对应靶点对接模式图，结果发现 Procyanidin b2、Daturic acid 及 Guanosine 与核心蛋白对接良好，表明筛选化合物可能直接结合蛋白质进而改变其功能，见图 9。进一步分析化合物与关键靶点的互作位置及相互作用方式，进而明确结合蛋白的关键位置，将得分最高化合物以多种构象化合物与蛋白对接，结果以热图呈现，见图 10。发现 TP53 的 GLU271、GLU285、ARG273、ARG249、SER249 的氨基酸位点，HIF1A 的 ALA34、HIS18、VAL148 位点以及 CA9 的 A200 位置可以与 Procyanidin b2 形成氢键，MAPK1 的 ARG351、ARG75 与 Daturic acid 形成氢键及静电相互作用，TSHR 的 PHE141、ASP143、ILE117、TYR116 与 Guanosine 形成氢键及碳氢键。结果表明上述蛋白氨基酸残基可能在与神秘果化合物结合过程中发挥重要作用。

3　讨论与结论

本实验探究神秘果干预肿瘤免疫进而辅助治疗肿瘤的可能性，通过液相-质谱联用技术及生物信息数据挖掘神秘果治疗肿瘤的潜在机制。发现神秘果干预肿瘤免疫过程中可能影响的主要靶点如下：TSHR 为促甲状腺激素受体，正常表达于甲状腺滤泡上皮细胞的细胞膜上，属于 G 蛋白偶联受体，其与 TSH 结合后被激活，进而激活 cAMP-PKA 信号通路，TSHR 的缺失可能会促进甲状腺乳头状癌的增殖、侵袭能力[42]。HIF1 为缺氧诱导因子-1，是细胞在缺氧状态下的关键调控蛋白，调节一系列细胞因子和生长介质的表达及蛋白质合成，对肿瘤血管的形成和对肿瘤细胞的增殖、转移、侵袭、凋亡、能量代谢等作用有着重要的影响[43]，HIF-1 的表达程度与肿瘤的恶性程度、新生血管的表达程度及预后不良呈正相关。抑制 HIF-1 的表达，阻断缺氧信号的传递，已经成为肿瘤治疗的新思路，而神秘果中有诸多成分，如

表 2　神秘果影响肿瘤免疫相关化合物影响信号通路

Table 2　*Synsepalum dulcificum* affects tumor immunity-related compounds affect signal pathways

序号	通路	通路 ID	*P*-value	富集基因数	基因组占比
1	Immune System	REACT:R-HSA-168256	0	1391	2118/44020 genes: 4.81%
2	Innate Immune System	REACT:R-HSA-168249	0	858	1298/44020 genes: 2.95%
3	Metabolic pathways	KEGG:hsa01100	0	890	1270/44020 genes: 2.89%
4	Metabolism	REACT:R-HSA-1430728	0	1536	2172/44020 genes: 4.93%
5	Metabolism of proteins	REACT:R-HSA-392499	0	984	1623/44020 genes: 3.69%
6	Signal Transduction	REACT:R-HSA-162582	0	1550	2588/44020 genes: 5.88%
7	Gene Expression	REACT:R-HSA-74160	2.16e-310	969	1831/44020 genes: 4.16%
8	Metabolism of lipids and lipoproteins	REACT:R-HSA-556833	6.33e -297	595	814/44020 genes: 1.85%
9	Developmental Biology	REACT:R-HSA-1266738	1.94e -270	672	1076/44020 genes: 2.44%
10	Cytokine Signaling in Immune system	REACT:R-HSA-1280215	9.13e -267	547	760/44020 genes: 1.73%

表 3 神秘果化合物对核心基因的影响

Table 3 Effects of *Synsepalum dulcificum* compounds on hub genes

核心基因	化合物	影响趋势	支持文献	实验物种
TP53	Choline	Choline results in decreased expression of TP53 mRNA	[13]	*Rattus norvegicus*
	Dopamine	[Dopamine results in increased expression of HIF1A protein] which results in increased expression of and results in increased phosphorylation of TP53 protein	[14]	*Homo sapiens*
	Glutathione	[Buthionine Sulfoximine results in decreased abundance of Glutathione] inhibits the reaction [Benzo(a)pyrene results in increased activity of TP53 protein]	[15]	*Homo sapiens*
	Glutathione	Dithiothreitol inhibits the reaction [Glutathione results in increased glutathionylation of TP53 protein]	[16]	*Homo sapiens*
	Glutathione	Glutathione inhibits the reaction [[CD40LG protein co-treated with IL4 protein] results in increased expression of TP53 protein]	[17]	*Homo sapiens*
	morin	morin inhibits the reaction [Ifosfamide results in increased expression of TP53 protein]	[18]	*Rattus norvegicus*
	morin	morin results in increased expression of TP53 mRNA	[19]	*Homo sapiens*
	myricetin	myricetin analog results in increased expression of TP53 protein	[20]	*Homo sapiens*
	myricitrin	myricitrin inhibits the reaction [Hydrogen Peroxide results in increased expression of TP53 protein]	[21]	*Homo sapiens*
	naringenin	naringenin results in increased expression of TP53 mRNA	[22]	*Homo sapiens*
	Quercetin	Quercetin results in decreased expression of TP53 protein	[23]	*Homo sapiens*
TSHR	Quercetin	Quercetin results in decreased expression of TSHR mRNA	[24]	*Rattus norvegicus*
MAPK1	Dopamine	Dopamine results in increased phosphorylation of and results in increased activity of MAPK1 protein	[25]	*Homo sapiens*
	Glutathione	Glutathione inhibits the reaction [Hydrogen Peroxide results in increased phosphorylation of MAPK1 protein]	[26]	*Canis lupus* familiaris
	morin	morin inhibits the reaction [Excitatory Amino Acid Agonists results in increased phosphorylation of MAPK1 protein]	[27]	*Rattus norvegicus*
	myricetin	myricetin results in decreased activity of MAPK1 protein	[28]	*Mus musculus*
	naringenin	naringenin results in decreased activity of MAPK1 protein	[29]	*Homo sapiens*
	naringenin	naringenin results in decreased phosphorylation of MAPK1 protein	[30]	*Homo sapiens*
	Palmitic Acid	Palmitic Acid results in increased phosphorylation of MAPK1 protein	[31]	*Homo sapiens*
	Sucrose	Sucrose results in decreased phosphorylation of and results in decreased activity of MAPK1 protein	[32]	*Mus musculus*
	Tyramine	Tyramine results in increased activity of MAPK1 protein	[33]	*Oryctolagus cuniculus*
HIF1A	Choline	[Methionine deficiency co-treated with Choline deficiency co-treated with Folic Acid deficiency] results in increased methylation of HIF1A gene	[34]	*Mus musculus*
	Dopamine	Dopamine results in decreased expression of HIF1A protein	[35]	*Rattus norvegicus*
	Dopamine	Dopamine results in increased expression of HIF1A protein	[14]	*Homo sapiens*
	Glutathione	Glutathione inhibits the reaction [15-deoxy-delta(12,14)-prostaglandin J2 results in increased expression of HIF1A protein]	[36]	*Homo sapiens*
	myricetin	myricetin results in decreased activity of HIF1A protein	[37]	*Homo sapiens*
	myricetin	myricetin results in increased expression of HIF1A protein	[38]	*Homo sapiens*
	Palmitic Acid	[Palmitic Acid co-treated with Oleic Acid co-treated with TNF protein] results in increased expression of HIF1A protein	[39]	*Homo sapiens*
	Quercetin	Quercetin inhibits the reaction [HIF1A protein binds to VHL protein]	[40]	*Homo sapiens*
	Quercetin	Quercetin results in decreased activity of HIF1A protein	[41]	*Homo sapiens*

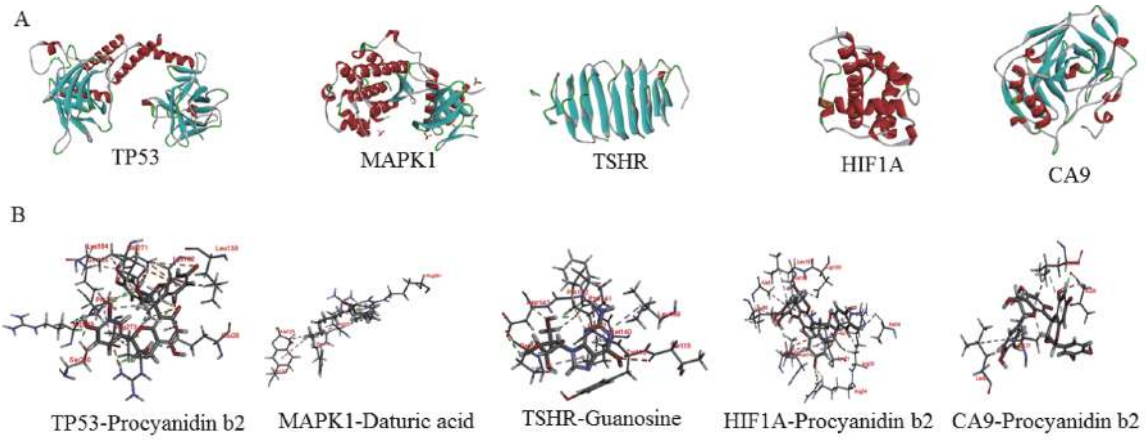


图 9 蛋白质与化合物对接图

Fig.9 Protein and compound docking diagram

注: A: 蛋白质三维结构图; B: 蛋白质与对接分数最高化合物的对接位置图。

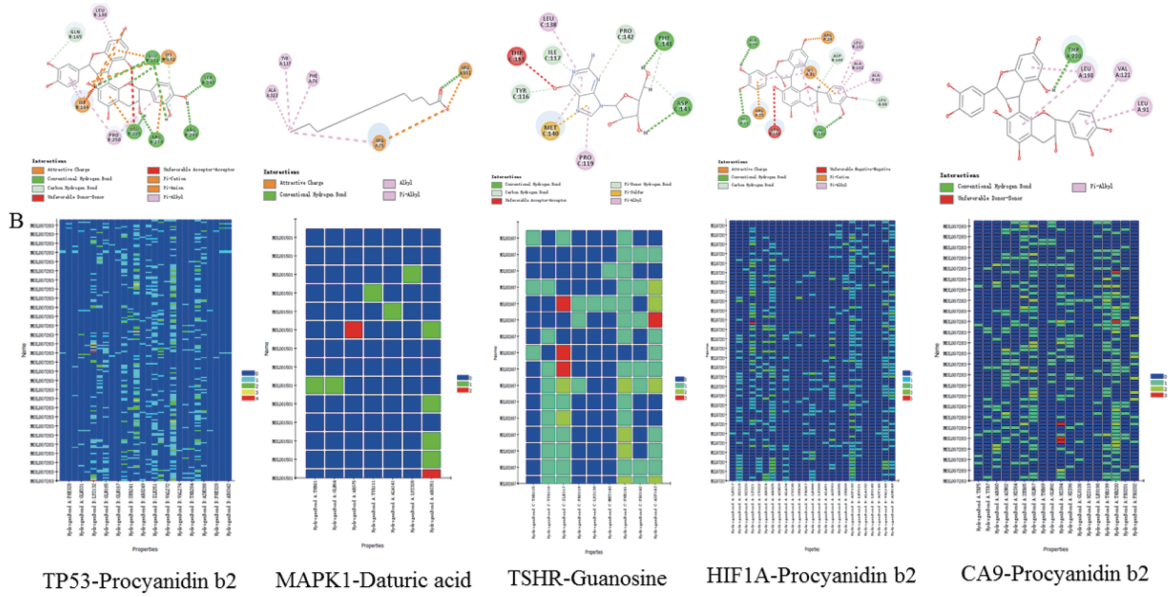


图 10　化合物与蛋白质相互作用图

Fig.10　Diagram of the interaction between compound and protein

注：A：蛋白质与化合物相互作用 2 维图；B：多种构象与蛋白质残基作用氢键热图。

杨梅素、槲皮素等均可影响靶点的表达。TP53 是肿瘤研究中热点靶点，有超过 50% 的肿瘤中存在 *TP*53 的基因突变，其中大多数都错义突变。突变型 p53 蛋白，不仅失去了野生型蛋白的肿瘤抑制功能，反而能促进肿瘤的发生和进展[44]。研究同时也发现神秘果含有抗肿瘤免疫成分 42 种，通过文献发现，部分的化合物对肿瘤免疫已有积极意义的报道。如槲皮素可以抑制淋巴细胞活化和增殖来发挥免疫调节作用。在对白血病细胞系的研究中，槲皮素对白血病细胞系的凋亡潜能高于外周血单核细胞，同时能够抑制 T 细胞增殖和活化等正常免疫功能[45]，同时槲皮素可诱导多种肿瘤细胞凋亡，如肝癌细胞[46]、肺癌[47]，槲皮素通过灭活 caspase/Cyto-c 途径、抑制 AP-23/h TERT、抑制 NF-κB/COX-2 和阻断 Akt/ERKl/2 信号通路而发挥抗肿瘤作用[48]。苦杏仁苷作为一种天然产生的氰化物，具有不影响正常细胞的情况下发挥抗肿瘤的作用，可通过直接抑制肾移植大鼠的免疫细胞的增殖进而干预免疫功能[49]，同样对影响膀胱癌细胞 (UMUC-3、RT112、TCCSUP) 的附着和迁徙[50]，通过 cDNA 微列分析发现苦杏仁甙能明显下调细胞周期相发挥抗肿瘤作用[51]。原花青素作为中药中广泛存在的多酚类化合物具有良好的抗肿瘤作用，可能通过增加线粒体膜通透性和细胞色素 C 从线粒体中释放及激活肿瘤细胞内的细胞凋亡蛋白酶和半胱天冬酶诱导肿瘤细胞凋亡，可抑制多种肿瘤细胞的生长[52]，例如抗黑素瘤[53] 及前列腺癌[54]。

综上，神秘果作为干预肿瘤的一种辅助治疗选择，是非常有潜力的，神秘果含有较多活性成分可以改善肿瘤患者的免疫水平进而影响肿瘤的发生发展。但以上部分实验仍为预测，需要继续实验验证以进一步确认结果，希望神秘果在肿瘤及免疫方面得到出色发挥，为抗肿瘤提供更多的选择。

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