二甲双胍抵抗 UVA 诱导皮肤光老化的转录组学分析

中文摘要

紫外线是外源性造成皮肤老化的主要原因之一。太阳光中的紫外线约 95%是波长在 315-400 nm 之间的长波紫外线（Ultraviolet A，UVA），UVA 穿透性强，可穿透皮肤的表皮层到达真皮层，严重将损伤皮肤深层造成皮肤光老化。皮肤光老化严重将引发日光性角化病和恶性黑色素瘤等皮肤病变。随着对皮肤光老化认识的加深，人们对皮肤光老化的预防与治疗越来越迫切。因此，深入分析皮肤光老化的转录组学差异，寻找抑制皮肤光老化的特效治疗药物尤为重要。本课题组前期发表的研究中显示，降血糖药二甲双胍具有显著的抑制皮肤光老化作用，该作用在人包皮成纤维细胞光老化模型、小鼠光老化模型、豚鼠光老化模型中均被证实，因此本研究将通过转录组学分析的方式继续深入挖掘二甲双胍抑制 UVA 诱导的皮肤光老化的靶基因，为 UVA 诱导的皮肤光老化的治疗提供新靶点。

实验目的：

1、尝试建立秀丽隐杆线虫的 UVA 光老化模型，并探讨其可行性。

2、利用前期建立的人包皮成纤维细胞光老化模型，开展转录组学分析，尝试从大数据角度深入挖掘二甲双胍抵抗 UVA 诱导皮肤光老化的作用机制。

实验方法：

1. 构建 N2 型秀丽隐杆线虫光老化模型，通过控制照射时间，设置了 5 种 UVA 照射剂量梯度，分别为 0、2.5、7.5、15、30 J/cm^2；

2. 每日观察记录每组秀丽隐杆线虫的死亡情况，并统计平均寿命和最长寿命；

3. DCFH-DA 染色评估每组秀丽隐杆线虫体内的 ROS 水平；

4. 荧光显微镜检测 UVA 照射对线虫体内脂褐素积累的影响；

5. trizol 法提取人包皮成纤维细胞的 RNA，利用转录组学测序的序列片段长度及 GC 含量比评估各组细胞样本质量；

6. 采用冗余序列分析、基因覆盖率统计和基因组结构分布的转录组学测序分

析对照组、UVA 组、二甲双胍组、UVA+二甲双胍组人包皮成纤维细胞的转录组差异；

7. 利用染色体测序序列分布分析 UVA 组、二甲双胍组、及 UVA 联合二甲双胍治疗组对人包皮成纤维细胞的影响；

8. 采用样本间的相关性分析、基因表达差异分析、差异基因注释与功能富集分析等对获得的转录组学数据进行分析，获得二甲双胍抑制 UVA 光老化的关键基因及信号通路。

实验结果：

1、秀丽隐杆线虫寿命统计结果显示，随着 UVA 照射剂量的增强线虫出现死亡的时间也逐渐提前，这提示秀丽隐杆线虫对 UVA 存在照射剂量敏感性。

2、ROS 检测结果显示，与对照组相比，在照射剂量为 0、2.5、7.5、15、30 J/cm^2 组中，秀丽隐杆线虫 ROS 水平显著升高，且具有照射剂量依赖性。在 UVA 照射剂量 30J/cm^2 组中，秀丽隐杆线虫体内 ROS 水平最高，表明 UVA 照射显著提高秀丽隐杆线虫体内的 ROS 水平，并呈照射剂量依赖性。

3、利用脂褐素堆积情况评估秀丽隐杆线虫光老化情况。结果显示，与对照组相比，7.5 J/cm^2 照射剂量组秀丽隐杆线虫体内的脂褐素水平明显增强，并且随着 UVA 照射剂量的增强，秀丽隐杆线虫体内的脂褐素水平显著增加。表明 UVA 诱导秀丽隐杆线虫体内脂褐素的积累，诱导其光老化的发生，并呈照射剂量依赖性。

4、转录组学测序结果显示，各组样本的总碱基数及片段长度符合要求，碱基质量在 20（Q20）以上的比例大于 98%，表明获得的测序序列质量达到转录组分析的质量要求。

5、对获得的碱基序列进行 GC 比含量分析发现，四组样本中的 GC 含量（GC%）均在 50%-52%之间，表明各组中的碱基差异分布正常。

6、冗余序列分析、基因覆盖率统计和基因组结构分布结果显示，各组样本冗余序列的含量及比例均正常。二甲双胍组 90%-100%被检测到的基因与各组基因数相差较大，表明二甲双胍对人包皮成纤维细胞的基因转录水平影响较大。基因组结构分析结果显示 UVA 照射及二甲双胍均会引起人包皮成纤维细胞的外显子数量的改变。

7、将各组获得的测序结果从染色体角度分析 UVA、二甲双胍对人包皮成纤维细胞的影响，结果提示 UVA 及二甲双胍对人包皮成纤维细胞均无染色体上的显著改变。

8、利用共表达韦恩图分析四组样本中的共有和独有的表达基因数量，显示共有 18268 个基因在四组样本中共表达，每组的特有差异表达基因均在 1000 个以上。采用 Heatmap 图评估四组样本间的相关性指数。结果显示，UVA+二甲双胍组与对照组间的相关性高于 UVA 组，提示二甲双胍在逆转 UVA 引起的光老化方面有一定作用。

9、差异基因表达分析结果显示，UVA+二甲双胍组基因表达差异较大，与对照组相比，共有 3828 个基因表达上调。提示二甲双胍从基因转录水平调控 UVA 对人包皮成纤维细胞的损伤作用。

10、利用蛋白互作网络分析结果显示，比较 UVA 组、UVA+二甲双胍组蛋白间的相互作用，ISY1-RAB43、BCL-2、SNX22 等显著上调，DAG1、MAP1S、GABARAPL1 等下调明显，UBC、ATM、MKI67 等的节点较大，说明上述蛋白在二甲双胍抑制 UVA 诱导的光老化过程中发挥着关键作用。

11、对获得的差异基因功能注释与功能富集分析差异基因功能。GO 富集分析分别从生物学过程、细胞组成、分子功能三个方面进行评估分析。比较 UVA+二甲双胍组与 UVA 组，纤维组装压力调节、光刺激反应、固醇反应、胆固醇反应以及调控 T 细胞反应等生物过程的一系列基因显著下调，而细胞基本组成包括细胞内部的基本结构成分、细胞外基质、细胞膜、DNA 结合及转录修复、MAPK 激酶活性等显著上调，表明二甲双胍能够明显抑制 UVA 对人包皮成纤维细胞的损伤，促进细胞结构的完整性修复，并降低细胞对 UVA 的刺激。KEGG 信号通路分析发现，神经活性配体受体相互作用、MAPK 信号通路、Jak-STAT 信号通路、Wnt 信号通路、Ras 信号通路等多条信号通路与二甲双胍抑制 UVA 诱导的皮肤光老化有关。

研究结论：

1、秀丽隐杆线虫光老化模型的成功制备，有效解决了传统动物光老化模型制备时间久、经济成本高的缺点，为研究光老化提供了新的实验方法。

2、转录组学分析了二甲双胍抵抗 UVA 照射引起人包皮成纤维细胞光老化的差异基因及表达，预测 MAPK 信号通路、Wnt 信号通路等在这其中的作用，为靶向开展皮肤光老化治疗及药物研发指明了方向。

关键词：

二甲双胍，UVA，光老化，氧化应激，秀丽隐杆线虫

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Abstract

Transcriptome analysis of metformin against UVA-induced skin photoaging

Ultraviolet radiation is one of the main causes of skin aging caused by exogenous factors. About 95% of the ultraviolet rays in sunlight are long wavelength ultraviolet A (UVA) with wavelengths between 315 and 400 nm. UVA has strong penetration ability and can penetrate the epidermal layer of the skin to reach the dermis, seriously damaging the deep layers of the skin and causing skin photoaging. Serious skin photoaging will lead to skin diseases such as actinic keratosis and malignant melanoma. With the deepening understanding of skin photoaging, the prevention and treatment of skin photoaging are becoming increasingly urgent. Therefore, it is particularly important to deeply analyze the transcriptome differences of skin photoaging and find specific therapeutic drugs to inhibit skin photoaging. The research team published earlier shows that the hypoglycemic drug metformin has a significant effect on inhibiting skin photoaging, which has been confirmed in human foreskin fibroblast photoaging model, mouse photoaging model, and guinea pig photoaging model. Therefore, this study will continue to explore the target genes of metformin inhibiting UVA induced skin photoaging through transcriptome analysis, Provide new targets for the treatment of UVA induced skin photoaging.

Purpose:

1. Establish a UVA photoaging model of *C. elegans* and explore its feasibility.

2. Using the previously established photoaging model of human foreskin fibroblasts, transcriptome analysis was conducted to explore the mechanism of metformin's resistance to UVA induced skin photoaging from the perspective of big data.

Methods:

1. Construct a photo aging model of N2 type *C. elegans*, control the irradiation time, and set 5 different UVA irradiation dose gradients, which are 0, 2.5, 7.5, 15, and 30 J/cm^2, respectively;

2. Daily observation and recording of the death status of each group of *C. elegans*, and statistics of the average and longest lifespan;

3. DCFH-DA staining was used to evaluate the ROS levels in each group of *C. elegans*;

4. Fluorescence microscope was used to detect the effect of UVA irradiation on lipofuscin accumulation in *C. elegans*;

5. The RNA of human foreskin fibroblasts was extracted by the trizol method, and the cell sample quality of each group was evaluated by the sequence fragment length and GC content ratio of transcriptome sequencing;

6. Redundant sequence analysis, gene coverage statistics and transcriptome sequencing of genome structure distribution were used to analyze the transcriptome differences of human foreskin fibroblasts in control group, UVA group, metformin group and UVA+metformin group;

7. Using chromosomal sequencing sequence distribution to analyze the effects of UVA group, metformin group, and UVA combined with metformin treatment group on human foreskin fibroblasts;

8. The obtained transcriptome data were analyzed by correlation analysis, gene expression difference analysis, differential gene annotation and function enrichment analysis among samples to obtain the key genes and signal pathways of metformin inhibiting UVA photoaging.

Results:

1. The statistical results of the lifespan of *C. elegans* show that the death time of the nematode gradually advances with the increase of UVA irradiation dose, indicating that *C. elegans* is sensitive to UVA irradiation dose.

2. The ROS detection results showed that compared with the control group, the ROS levels of *C. elegans* were significantly increased in the irradiation dose groups of 0, 2.5, 7.5, 15, and 30 J/cm2, and there was a dose-dependent effect. In the group with a UVA irradiation dose of 30J/cm^2, the ROS level in the body of *C. elegans* was the highest,

indicating that UVA irradiation significantly increased the ROS level in the body of *C. elegans* in a dose-dependent manner.

3. The accumulation of lipofuscin was used to evaluate the photoaging of *C. elegans*. The results showed that compared with the control group, the level of lipofuscin in the 7.5 J/cm^2 irradiation group was significantly increased, and with the increase of UVA irradiation dose, the level of lipofuscin in the *C. elegans* was significantly increased. The results showed that UVA induced the accumulation of lipofuscin in C. elegans and the occurrence of photoaging in a dose-dependent manner.

4. The transcriptome sequencing results show that the total base number and fragment length of each group of samples meet the requirements, and the proportion of base quality above 20 (Q20) is more than 98%, indicating that the quality of the sequencing sequence obtained meets the quality requirements of transcriptome analysis.

5. GC content analysis of the obtained base sequences revealed that the GC content (GC%) in all four groups of samples ranged from 50% to 52%, indicating a normal distribution of base differences among the groups.

6. The results of redundant sequence analysis, gene coverage statistics, and genome structure distribution showed that the content and proportion of redundant sequences in each group of samples were normal. The significant difference in the number of genes detected between the 90% -100% metformin group and each group indicates that metformin has a significant impact on the gene transcription level of human foreskin fibroblasts. The results of genomic structure analysis showed that both UVA irradiation and metformin can cause changes in the number of exons in human foreskin fibroblasts.

7. The sequencing results obtained from each group were analyzed from a chromosomal perspective to investigate the effects of UVA and metformin on human foreskin fibroblasts. The results showed that both UVA and metformin showed no significant chromosomal changes in human foreskin fibroblasts.

8. Analyzing the number of common and unique expression genes in the four groups of samples using co expression Wayne diagrams, it was found that a total of 18268 genes were co expressed in the four groups of samples, with each group having over 1000 unique differentially expressed genes. Evaluate the correlation index between the four

groups of samples using the Heatmap map. The results showed that the correlation between the UVA+metformin group and the control group was higher than that of the UVA group, indicating that metformin has a certain role in reversing UVA induced photoaging.

9. The results of differential gene expression analysis showed that there was a significant difference in gene expression in the UVA+metformin group, with a total of 3838 genes upregulated compared to the control group. It suggests that metformin regulates the damage effect of UVA on human foreskin fibroblasts at the gene transcription level.

10. The results of protein interaction network analysis showed that comparing the interactions between UVA group and UVA+metformin group proteins, ISY1-RAB43, BCL-2, SNX22, etc. were significantly upregulated, while DAG1, MAP1S, GABARAPL1, etc. were significantly downregulated. The nodes of UBC, ATM, MKI67, etc. were larger, indicating that the above proteins play a key role in the inhibition of UVA induced photoaging by metformin.

11. Functional annotation and enrichment analysis of differentially expressed genes obtained. GO enrichment analysis is evaluated and analyzed from three aspects: biological processes, cell composition, and molecular function. Comparing the UVA+metformin group with the UVA group, a series of genes involved in biological processes such as fiber assembly pressure regulation, light stimulation response, sterol response, cholesterol response, and regulation of T cell response were significantly downregulated, while the basic cellular composition, including basic structural components within the cell, extracellular matrix, cell membrane, DNA binding and transcriptional repair, MAPK kinase activity, was significantly upregulated, This indicates that metformin can significantly inhibit the damage of UVA to human foreskin fibroblasts, promote the integrity repair of cell structure, and reduce the stimulation of cells to UVA. KEGG signaling pathway analysis found that multiple signaling pathways, including neuroactive ligand receptor interactions, MAPK signaling pathway, Jak STAT signaling pathway, Wnt signaling pathway, and Ras

signaling pathway, are associated with metformin inhibiting UVA induced skin photoaging.

Conclusion:

1. The successful preparation of the photoaging model of the *C. Elegans* effectively solves the shortcomings of traditional animal photoaging models, such as long preparation time and high economic cost, and provides a new experimental method for studying photoaging.

2. Transcriptome analyzed the differential genes and expression of metformin resistance to UVA irradiation induced photoaging of human foreskin fibroblasts, predicted the role of MAPK signaling pathway, Wnt signaling pathway, etc. in this, and pointed out the direction for targeted skin photoaging treatment and drug development.

Keywords:

Metformin, UVA, photoaging, oxidative stress, *C. elegans*.

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