Blue Light Aminolevulinic Acid Photodynamic Therapy Downregulates Cell Division and Proliferation Pathways in Cutaneous Squamous Cell Carcinoma

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Abstract

5-Aminolevulinic (5-ALA) acid photodynamic therapy (PDT) is a treatment for actinic keratosis and has been studied as a treatment for non-invasive cutaneous squamous cell carcinoma (cSCC). PDT induces apoptosis and necrosis in actinic keratosis and cSCC. 5-ALA blue light PDT may modulate gene expression and pathways in surviving cells. In this study, differential gene expression and pathway analysis of cSCC and human dermal fibroblasts were compared before and after 5-ALA blue light PDT using RNA-Sequencing. No genes were differentially expressed after correcting for multiple testing (FDR <0.05). As a result, transcription factor, gene enrichment, and pathway analysis were performed with genes identified before multiple testing ($p<0.05$). Pathways associated with proliferation and carcinogenesis were downregulated. These findings using 5-ALA blue light PDT are similar to previously published studies using methyl-aminolevulinic and red light protocols, indicating that surviving residual cells may undergo changes consistent with a less aggressive cancerous phenotype.

Introduction

Cutaneous squamous cell carcinoma (cSCC) is the second most common cancer in the United States, behind basal cell carcinoma (BCC).[1-4] cSCC is also the second most likely skin cancer to metastasize, following melanoma.[1-4]Globally, 2.4 million cases and 56,100 deaths were attributable to cutaneous cSCC in 2019.[5] Actinic keratoses (AKs) are precancerous skin lesions that can potentially progress to SCC. AKs and cSCC represent aberrant keratinocytes, with AKs characterized by a less aggressive phenotype. AKs are the most commonly diagnosed pathology in dermatology and have an estimated prevalence of 11.5 to 26% in the United States.[6,7] Treating AKs is important as progression to SCC represents a significant public health risk.[1-4,8] From 2007 to 2011, the cost of treating nonmelanoma skin cancer, including SCC and BCC, totaled over 4.5 billion US dollars.[8]

5-Aminolevulinic acid (5-ALA) photodynamic therapy (PDT) is a Federal Drug Administration (FDA) approved treatment for AK and has been studied in the management of non-invasive SCC and BCC tumors.[9-12] During PDT, 5-ALA is either applied to single lesions or to a field of skin that includes aberrant and surrounding normal cells. 5-ALA is then metabolized to photosensitive protoporphyrin IX (PP-IX).[13,14] Cancerous cell types have decreased metabolization of PP-IX compared to normal cells, resulting in preferential accumulation.[13-16] PP-IX absorbs blue light (˜417 nm) from 1000 seconds of phototherapy, producing reactive oxygen species and subsequent cell death.[14]

PDT leads to the targeted destruction of cSCC cells by inducing apoptosis and necrosis.[13,14,17] However, a residual population of cells may not undergo cell death.[18] In these cells, 5-ALA blue light PDT may induce favorable changes in gene expression to a more benign profile. Previous research has shown that PDT treatment of AKs with methyl-aminolevulinic acid (MAL) and red light may beneficially alter the expression

of cancer genes.[19-22] MAL PDT has been shown to downregulate genes and pathways related to cellular proliferation while upregulating keratinocyte differentiation markers and autophagy.[19-22] The present study investigates whether 5-ALA blue light PDT similarly modulates gene expression and pathways related to proliferation and cancer pathogenesis in cSCC cells that survived PDT. Differential gene expression and pathway analysis of cSCC and human dermal fibroblasts (HDFs) were compared before and after 5-ALA blue light PDT using RNA-Sequencing (RNA-Seq).

Methods

Culture **:** Two cSCC cell lines (SCC-13 and A431) and two HDFs cell lines (AG13145 and CRL-2617) were cultured according to existing protocols.[13,18,23] cSCCs and HDFs were studied to evaluate the cellular composition of a skin biopsy representative of AK/cSCC.

5-ALA Treatment : Twenty-four hours following plating, cSCC cell lines were treated with 0 or 0.5 mM 5-ALA (SUN pharmaceuticals) and incubated for thirty minutes on a custom-designed heating block at temperatures of 39 °C, according to existing protocols.[13,18,23] Thermal incubation of 5-ALA enhances treatment efficacy in vitro.[18]

Blue Light Photoactivation **:** Cells were irradiated using a commercially available blue light device (BLU-U, SUN Pharmaceuticals) as per prior protocols.[13,18,23] The blue light device produces a uniform field of blue light (420 ± 5 nm) on a black surface. The blue light has a power density of 10 W/cm^2 at 5 cm. After 1000 seconds of irradiation with blue light, the total fluence was 10 J/cm^2.

RNA Collection : At 0, 4, and 24 hours following blue light photoactivation, RNA was purified using miRNeasy Kit (Qiagen) with centrifuge and Qiashredder (Qiagen) extraction method, according to manufacturers' protocols. The RNase-free DNA digestion set (Qiagen) included a DNA Lysis step (Qiagen). RNA concentrations and 260/280 scores were quantified using a Nanodrop One spectrophotometry (Thermofisher Scientific). Isolated RNA was stored at -80°C and submitted to the Icahn School of Medicine at Mount Sinai Genomics Core Facility for RNA-Seq.

RNA sequencing and bioinformatics : Libraries were prepared for RNA RNA-Seq using the Stranded Total RNA with RiboZero Plus library prep kit (Illumina). RNA-Seq was performed using NovaSeq 6000 SP Reagent Kit v1.5 (300 cycles) to achieve about 26 million reads per sample and paired-end 150bp (Illumina). Q30 and quality score are included in Supplemental Table 1.

Bioinformatics : Principal component analysis (PCA) was applied to the normalized samples to determine how samples cluster across cell lines, time points, and treatments. Differentially expressed genes between ALA-PDT and control samples were computed using the limma-voom.[24] The limma-voom gene sets were generated by sorting the results using limma's moderated t-statistic. From the sorted results, we analyzed the most significant up- and down-regulated gene sets with a p-value of < 0.05 without multiple hypotheses correction with the BH method. After BH correction, no statistically significant differentially expressed genes were identified. Enrichment analysis was performed using Enrichr against the GO Biological Processes 2021, KEGG 2021, ENCODE, and ChEA 2022 gene set libraries.[25,26]

Results

Principle component and heat maps

PCA revealed that the HDFs, AG13145 and CRL-2617, clustered separately from the cSCC cell lines, SCC-13 and A431 (Figure 1A). Additionally, there was greater clustering among cell lines than between treatment and control (Figure 1B). Heat map analysis demonstrated similar clustering of treatment and control groups among fibroblast and cSCC cells (Figure 1C).

Differential gene expression and enrichment analysis

No genes were identified that were differentially expressed with a false discovery rate (FDR) less than 0.05 following PDT in the pooled analysis of the fibroblast and cSCC cell lines (Supplemental Table 2). The FDR

is an adjusted p-value after correcting for multiple testing. As a result, transcription factor, gene enrichment, and pathway analysis were performed with genes with a p-value less than 0.05. Eighty-three genes were identified to be upregulated and 134 were found to be downregulated (Figure 1D).

Pathways related to cell division and proliferation were found to be downregulated. The specific pathways that were significantly downregulated with an FDR value <0.05 in KEGG 2021 included "Cell Cycle" (Figure 2A). GO Biological Processes 2021 pathways that were downregulated include microtubule cytoskeleton organization involved in mitosis (GO:1902850), mitotic spindle organization (GO:0007052), and mitotic nuclear division (GO:0140014) (Figures 2C). Supplemental Table 3 includes the full list of downregulated GO Biological Processes 2021. No KEGG 2021 or GO Biological Processes 2021 were significantly enriched (FDR < 0.05) in the upregulated genes (Figures 2B and 2D). KEGG 2021 pathways with a p <0.05 include mitophagy (FDR=0.21) and autophagy (FDR=0.33).

Transcription factors were analyzed using consensus genes targets in the ENCODE and ChEA 2022 databases. Following ALA PDT, 23 transcription factors were significantly enriched in downregulated genes (Supplemental Table 4). The transcription factors with the most significant enrichment included E2F4, FOXM1, Sin3a, and NFYA/B (Figure 3A). Figure 3C demonstrates the clustergram of genes commonly downregulated among the enriched transcription factors in the ENCODE and ChEA databases. Upregulated transcription factors included CPEBP (FDR=0.60) and NFE2L2 (FDR=0.29) but were not significant (Figures 3C and D).

Discussion

PDT is FDA approved treatment for actinic keratosis, and existing clinical research indicates that PDT may be effective in treating Bowen Disease, a type of primary cSCC.[11,12] Previous laboratory research demonstrated that 5-ALA PDT significantly increases cell apoptosis in cSCC and fibroblast cells treated with the same parameters used in this protocol.[18,27] The goal was to determine the genes and pathways altered in the residual population of ALA PDT treated cells that did not undergo apoptosis or other forms of cell death. RNA-Seq was performed using RNA from cSCC and fibroblast cells collected at 0-, 4-, and 24 hours following PDT. Statistical analysis did not identify any genes that were differentially expressed after correcting for multiple testing (FDR < 0.05). Transcription factor and pathway analysis were compared with differentially expressed genes with a p-value < 0.05. Bioinformatic analysis identified key transcription factors and pathways related to cellular proliferation and cancer pathogenesis were downregulated.

The findings of this RNA-Seq experiment conform to previous microarray and transcriptomic studies using PDT on AKs and cSCCs. Joly et al. compared differential expression in cSCC lesional, perilesional, and unexposed skin in immunocompromised patients up to 18 weeks following MAL PDT treatment.[19] MAL PDT downregulated cancer and cell proliferation genes, while extracellular matrix-associated genes were upregulated.[19] MAL PDT downregulated genes associated with proliferation, such as cyclins and the Kinesin Family member (KIF) proteins, were similarly identified in our testing with a p <0.05. KIF proteins are involved with microtubule organization during mitosis and have been previously found to be associated with cancer.[28-31] Knockdown of KIF22 inhibits tongue SCC proliferation and xenograft tumor growth.[28] In laboratory studies, PDT treatment protocols decreased cSSC tumor proliferation and increased apoptosis and autophagy via regulation of MAPK protein, particularly AKT.[20-22] Compared to the present study, autophagy was one of upregulated KEGG 2021 pathways with a p < 0.05, but FDR > 0.05.

Transcription factors associated with cancer proliferation and pathogenesis and known to be upregulated in cSCC, including E2F4 and FOXM1, were found in our study to be downregulated post ALA PDT.[32-34] The E2F family of transcription factors bind to DNA and Rb and regulate the cell cycle.[32] E2F4 is a transcriptional activator. Upregulation of E2F transcription factors has been linked to cancer progression in head and neck SCC.[32] FOXM1 is an ultraviolet master regulator and is upregulated in cSCC.[33,35,36] Inhibition of FOXM1 with thiostrepton and CRISPR depletion led to decreased cell viability and proliferation in cSCC.[33,35,36]

Limitations of this study include the lack of abundant commercially available cSCC cell lines at the onset

of this study. Due to the inability to significantly identify differential expression of individual genes with an FDR <0.05, the pathway analysis may indicate that small changes in gene expression may result in changes in pathways. This could be addressed by increasing the number of cell lines tested, and could be performed in future experiments. For RNA-Seq, there is no consensus regarding the number of biological replicates with n ranging from 3 to 12.[37] 4 samples were used, but the cell types differed between normal (i.e., HDF) and cancerous (i.e., cSCC). In the future, it may be necessary to test additional biological replicates and specifically increase the number of cSCC cell lines. Historically, there were a limited number of commercially available cSCC cell lines. However, multiple cSCC from transplant/immunocompromised and immunocompetent donors have recently become available. Several other potential explanations may explain why the PDT treatment did not result in significant differential expression after multiple testing, including dose response and genetic heterogeneity among the cell types. The PCA and heatmaps in Figure 1A demonstrate clustering among the two fibroblast cell lines, AG13145 and CRL-2617, and heterogeneity between the cSCC cell lines, SCC-13 and A431. Additionally, there were greater differences between samples than between treatment and control (Figure 1B), indicating that small, but potentially meaningful changes occur in the individual cell lines post ALA PDT.

In conclusion, RNA-Seq analysis of cSCC and HDF differential expression after ALA PDT did not identify any significant genes after correcting for multiple testing. However, pathways associated with proliferation and carcinogenesis were downregulated. These findings using ALA PDT are similar to previously published studies using MAL PDT that indicate that residual cells post PDT undergo changes consistent with a less aggressive cancerous phenotype.[19,20] Additional laboratory and clinical studies need to be performed to confirm the efficacy of ALA PDT for cSCC.

Figures

Figure 1. PCA and heatmap analysis of RNA-Seq. A) PCA of the SCC-13, A431, AG13145, and CRL-2617 following PDT color-coded by cell type. B) PCA of 4 sample cell lines color-coded by treatment; red as control and blue as ALA PDT. C) Heatmap analysis of SCC-13, A431, AG13145, and CRL-2617 following PDT. D) Set of genes with p-value < 0.05 used for pathway analysis with red as upregulated and blue as downregulated genes. No genes were differentially expressed when multiple testing was performed (FDR < 0.05).

Figure 2. KEGG 2021 and GO 2021 pathway analysis A) down- and B) upregulated pathways using KEGG 2021 gene set. C) Down- and D) upregulated pathways using GO 2021 gene set. The ten pathways with the lowest p-value are demonstrated. No individual genes were differentially expressed with an FDR<0.05. Pathway analysis used genes with p<0.05, which does not account for multiple testing.

Figure 3. Transcription factors regulators of genes from consensus ENCODE and ChEA datasets. A) down- and B) upregulated transcription factors. C) Heatmap of down- and D) upregulated pathways demonstrating common genes among enriched transcription factors. No individual genes were differentially expressed with an FDR<0.05. Transcription factor analysis was performed using genes with p<0.05, which does not account for multiple testing.

1. Combalia A, Carrera C. Squamous Cell Carcinoma: An Update on Diagnosis and Treatment. *Dermatol Pract Concept* . Jul 2020;10(3):e2020066. doi:10.5826/dpc.1003a66

2. Kauvar AN, Arpey CJ, Hruza G, Olbricht SM, Bennett R, Mahmoud BH. Consensus for Nonmelanoma Skin Cancer Treatment, Part II: Squamous Cell Carcinoma, Including a Cost Analysis of Treatment Methods.*Dermatol Surg* . Nov 2015;41(11):1214-40. doi:10.1097/dss.0000000000000478

3. Lewis KG, Weinstock MA. Trends in nonmelanoma skin cancer mortality rates in the United States, 1969 through 2000. *J Invest Dermatol* . Oct 2007;127(10):2323-7. doi:10.1038/sj.jid.5700897

4. Rogers HW, Weinstock MA, Harris AR, et al. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. *Arch Dermatol* . Mar 2010;146(3):283-7. doi:10.1001/archdermatol.2010.19

5. Zhang W, Zeng W, Jiang A, et al. Global, regional and national incidence, mortality and disability-adjusted life-years of skin cancers and trend analysis from 1990 to 2019: An analysis of the Global Burden of Disease Study 2019. *Cancer Med* . Jul 2021;10(14):4905-4922. doi:10.1002/cam4.4046

6. Reinehr CPH, Bakos RM. Actinic keratoses: review of clinical, dermoscopic, and therapeutic aspects. *An Bras Dermatol* . Nov-Dec 2019;94(6):637-657. doi:10.1016/j.abd.2019.10.004

7. Salasche SJ. Epidemiology of actinic keratoses and squamous cell carcinoma. *J Am Acad Dermatol* . Jan 2000;42(1 Pt 2):4-7. doi:10.1067/mjd.2000.103342

8. Guy GP, Jr., Machlin SR, Ekwueme DU, Yabroff KR. Prevalence and costs of skin cancer treatment in the U.S., 2002-2006 and 2007-2011. *Am J Prev Med* . Feb 2015;48(2):183-187. doi:10.1016/j.amepre.2014.08.036

9. Morton C, Szeimies RM, Sidoroff A, et al. European Dermatology Forum Guidelines on topical photodynamic therapy. *Eur J Dermatol* . Jul-Aug 2015;25(4):296-311. doi:10.1684/ejd.2015.2570

10. Saini R, Lee NV, Liu KY, Poh CF. Prospects in the Application of Photodynamic Therapy in Oral Cancer and Premalignant Lesions.*Cancers (Basel)* . Sep 2 2016;8(9)doi:10.3390/cancers8090083

11. Morton CA, Szeimies RM, Basset-Seguin N, et al. European Dermatology Forum guidelines on topical photodynamic therapy 2019 Part 1: treatment delivery and established indications - actinic keratoses, Bowen's disease and basal cell carcinomas. *J Eur Acad Dermatol Venereol* . Dec 2019;33(12):2225-2238. doi:10.1111/jdv.16017

12. Zeitouni NC, Bhatia N, Ceilley RI, et al. Photodynamic Therapy with 5-aminolevulinic Acid 10% Gel and Red Light for the Treatment of Actinic Keratosis, Nonmelanoma Skin Cancers, and Acne: Current Evidence and Best Practices. *J Clin Aesthet Dermatol* . Oct 2021;14(10):E53-e65.

13. Austin E, Jagdeo J. An In Vitro Approach to Photodynamic Therapy.*J Vis Exp* . Aug 17 2018;(138)doi:10.3791/58190

14. Kwiatkowski S, Knap B, Przystupski D, et al. Photodynamic therapy - mechanisms, photosensitizers and combinations. *Biomed Pharmacother* . Oct 2018;106:1098-1107. doi:10.1016/j.biopha.2018.07.049

15. Josefsen LB, Boyle RW. Photodynamic therapy and the development of metal-based photosensitisers. *Met Based Drugs* . 2008;2008:276109. doi:10.1155/2008/276109

16. Ohgari Y, Nakayasu Y, Kitajima S, et al. Mechanisms involved in delta-aminolevulinic acid (ALA)-induced photosensitivity of tumor cells: relation of ferrochelatase and uptake of ALA to the accumulation of protoporphyrin. *Biochem Pharmacol* . Dec 19 2005;71(1-2):42-9. doi:10.1016/j.bcp.2005.10.019

17. Rkein AM, Ozog DM. Photodynamic therapy. *Dermatol Clin* . Jul 2014;32(3):415-25, x. doi:10.1016/j.det.2014.03.009

18. Austin E, Koo E, Jagdeo J. Thermal photodynamic therapy increases apoptosis and reactive oxygen species generation in cutaneous and mucosal squamous cell carcinoma cells. *Sci Rep* . Aug 22 2018;8(1):12599. doi:10.1038/s41598-018-30908-6

19. Joly F, Deret S, Gamboa B, et al. Photodynamic therapy corrects abnormal cancer-associated gene expression observed in actinic keratosis lesions and induces a remodeling effect in photodamaged skin. *J Dermatol Sci* . May 17 2018;doi:10.1016/j.jdermsci.2018.05.002

20. Zeng Q, Liu J, Yan Y, et al. Modified 5-aminolevulinic acid photodynamic therapy suppresses cutaneous squamous cell carcinoma through blocking Akt/mTOR-mediated autophagic flux. *Front Pharmacol* . 2023;14:1114678. doi:10.3389/fphar.2023.1114678

21. Liu J, Yan G, Chen Q, Zeng Q, Wang X. Modified 5-aminolevulinic acid photodynamic therapy (M-PDT) inhibits cutaneous squamous cell carcinoma cell proliferation via targeting PP2A/PP5-mediated MAPK signaling pathway. *Int J Biochem Cell Biol* . Aug 2021;137:106036. doi:10.1016/j.biocel.2021.106036

22. Zeng Q, Zhou C, Zhang Y, Yan G, Wang X. Modified 5-aminolevulinic acid photodynamic therapy reduces pain and improves therapeutic effects in cutaneous squamous cell carcinoma mouse model. *Lasers Surg Med* . Jul 2022;54(5):804-812. doi:10.1002/lsm.23516

23. Austin E, Koo E, Merleev A, et al. Transcriptome analysis of human dermal fibroblasts following red light phototherapy. *Sci Rep* . Apr 1 2021;11(1):7315. doi:10.1038/s41598-021-86623-2

24. Law CW, Chen Y, Shi W, Smyth GK. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol* . Feb 3 2014;15(2):R29. doi:10.1186/gb-2014-15-2-r29

25. Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res* . Jul 8 2016;44(W1):W90-7. doi:10.1093/nar/gkw377

26. The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Res* . Jan 8 2021;49(D1):D325-d334. doi:10.1093/nar/gkaa1113

27. Koo E, Austin E, Mamalis A, Jagdeo J. Thermal Ultra Short Photodynamic Therapy: Heating Fibroblasts During Sub-30-Minute Incubation of 5-Aminolevulinic Acid Increases Photodynamic Therapy-Induced Cell Death. *Dermatol Surg* . Apr 2018;44(4):528-533. doi:10.1097/dss.0000000000001341

28. Liu Y, Li R-H, Ren G, Jiang J. Suppression of KIF22 inhibits cell proliferation and xenograft tumor growth in tongue squamous cell carcinoma. *BioMed Research International* . 2020;2020

29. Zhang X, Wu M, Peng G, et al. Aberrant kinesin family member 2A signifies tumor size and invasion, and may help predict prognosis of patients with papillary thyroid carcinoma. *Oncol Lett* . Aug 2022;24(2):256. doi:10.3892/ol.2022.13376

30. Zhong Y, Jiang L, Lin H, et al. Overexpression of KIF18A promotes cell proliferation, inhibits apoptosis, and independently predicts unfavorable prognosis in lung adenocarcinoma. *IUBMB Life* . Jul 2019;71(7):942-955. doi:10.1002/iub.2030

31. Zuo X, Meng P, Bao Y, et al. Cell cycle dysregulation with overexpression of KIF2C/MCAK is a critical event in nasopharyngeal carcinoma. *Genes Dis* . Jan 2023;10(1):212-227. doi:10.1016/j.gendis.2021.05.003

32. Li Y, Huang Y, Li B, Yang K. Roles of E2F family members in the diagnosis and prognosis of head and neck squamous cell carcinoma.*BMC Medical Genomics* . 2023;16(1):1-14.

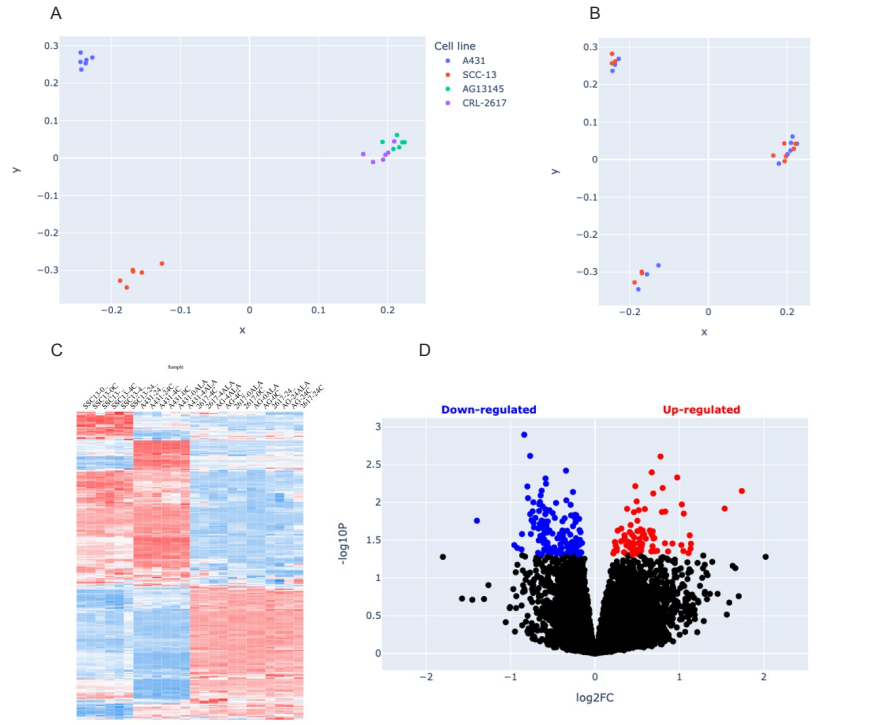
33. Teh MT, Gemenetzidis E, Chaplin T, Young BD, Philpott MP. Upregulation of FOXM1 induces genomic instability in human epidermal keratinocytes. *Mol Cancer* . Feb 26 2010;9:45. doi:10.1186/1476-4598-9-45

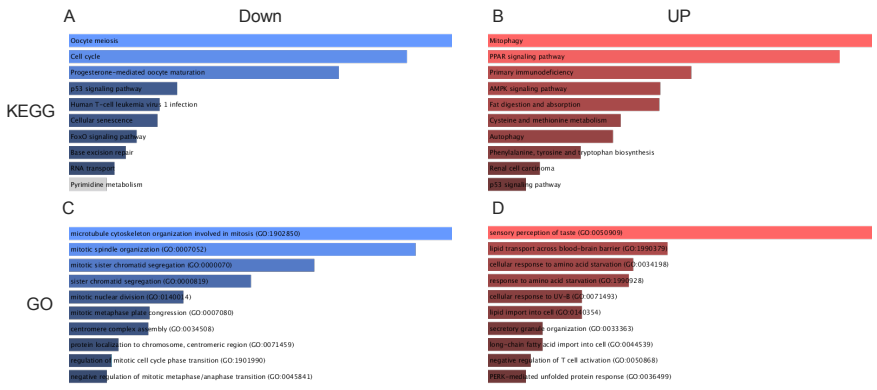
34. McHugh A, Fernandes K, Chinner N, et al. The identification of potential therapeutic targets for cutaneous squamous cell carcinoma.*Journal of Investigative Dermatology* . 2020;140(6):1154-1165. e5.

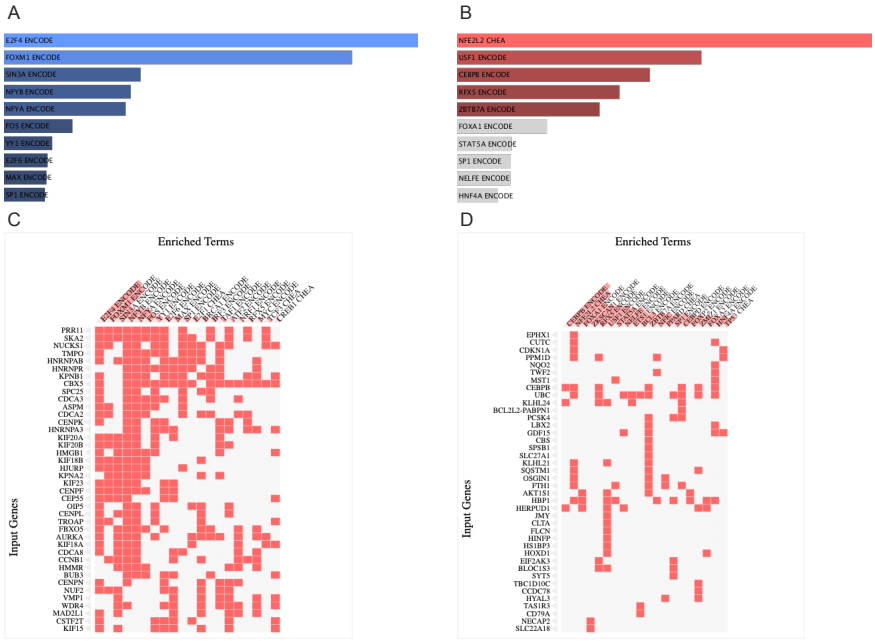
35. Ha W, Hinde A, Xie L, Trager MH, Liu L. Biomarker function of HMGA2 in ultraviolet-induced skin cancer development. *Exp Dermatol* . Oct 2020;29(10):1021-1026. doi:10.1111/exd.14174

36. Shen Y, Chan G, Xie M, Zeng W, Liu L. Identification of master regulator genes of UV response and their implications for skin carcinogenesis. *Carcinogenesis* . Jul 4 2019;40(5):687-694. doi:10.1093/carcin/bgy168

37. Schurch NJ, Schofield P, Gierliński M, et al. How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? *Rna* . 2016;22(6):839-851.







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