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Temporal Regulation of a Cellular Stress Response Transcriptional Program in a Murine Model of Radiation-Induced Skin Injury

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ABSTRACT

Radiation-induced skin injury encompasses moderate to severe cutaneous complications of radiation exposure, with an incidence of 90% among patients undergoing radiotherapy and a negative impact on quality of life. To elucidate the temporal genomic signature of radiation wounding in the skin, mice were treated with 30Gy radiation delivered in fractionated doses over a 12-day period, with non-irradiated age- and sex-matched mice serving as controls. RNA sequencing was performed on irradiated and control skin at 1 week and 4 weeks post-treatment (n=5 mice per control/irradiated group per time point). By Ingenuity Pathway Analysis, top enriched pathways post-radiation involved cellular stress responses, including senescence (p=3.4 x 10^{-12}), DNA damage (p=3.6 x 10^{-7}), *p53* activation (p=2.57 x 10^{-4}), and sonic hedgehog signaling (p=1.5 x 10^{-6}). Differentially expressed genes belonging to multiple stress response pathways (*Brca1*, *Mybl2*, *Ptch2*, *Cdk1*, *Shh*) were selected for further validation by qPCR in an expanded group of mice. Interestingly, these genes were downregulated in the skin 1 week post-irradiation (*Mybl2* p=0.024, fold change (FC)=-2.71; *Ptch2* p=0.022, FC=-9.7), but then upregulated at Week 4 (*Mybl2* p=0.004, FC=+3.6; *Brca1* p=0.002, FC=+5.4; *Cdk1* p=0.0019, FC=+6.01)), suggesting a biphasic cellular stress response. Further studies are indicated to determine the skin structures and cell types driving this transcriptomic response in the weeks following radiation. Further understanding of the cellular stress wounding response after radiation can pave the way for early therapeutic interventions to mitigate cutaneous complications and improve clinical outcomes.

BACKGROUND

Radiation-induced skin injury is a devastating consequence of radiation exposure and develops as a consequence of aberrant wound healing.

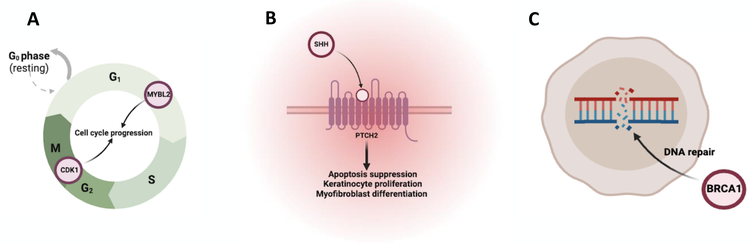


Figure 1. (A) Cell cycle checkpoints: CDK1 enzyme controls progression from G2 to M phase and transcription factor MYBL2 controls progression from G1 to S phase **(B)** SHH ligand binds to PTCH2 receptor promoting keratinocyte and myofibroblast differentiation and proliferation **(C)** BRCA1 is a key DNA repair protein that prevents genomic instability

Radiation-induced skin injury triggers a cascade of acute responses, including inflammation and oxidative stress, which act synergistically to amplify tissue damage **(Figure 1).** In an effort to counteract these effects, cells activate a broad stress response program that addresses disorganized angiogenesis, extracellular matrix disruption, DNA damage, and apoptosis—processes that, if unresolved, can culminate in chronic remodeling and fibrosis (**Figure 2**).

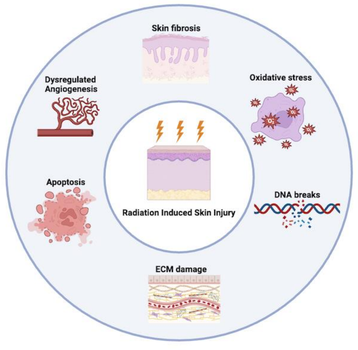
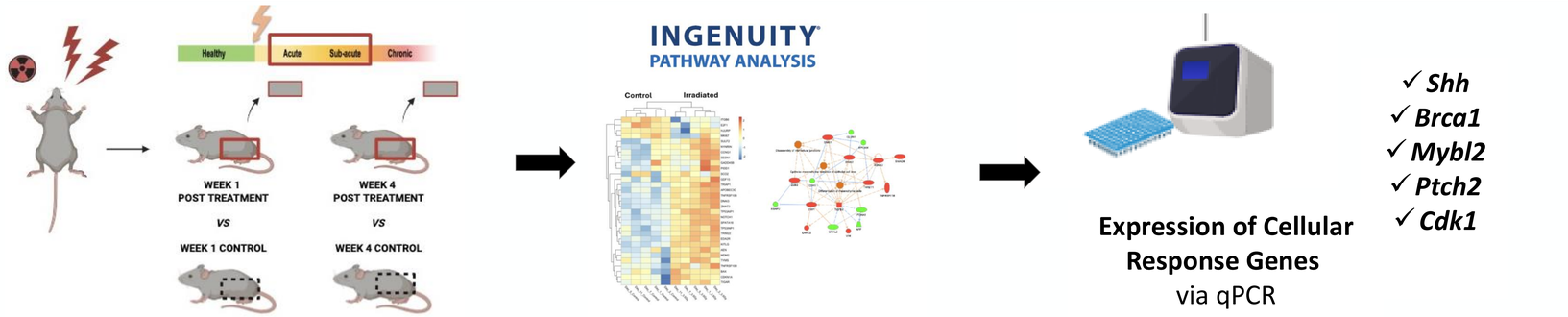


Figure 2. Pathogenesis of radiation induced skin injury

METHODS



Skin from mice (n=5 per group per time point) was harvested from irradiated and age- and sex-matched non-irradiated controls. Irradiated mice received a total dose of **30 Gy**, delivered in **fractionated doses** over a **12-day period**. Full-thickness skin was collected at **1 week** and **4 weeks post-radiation** for **RNA sequencing**. Differential gene expression analysis and **Ingenuity Pathway Analysis** identified significant enrichment of cellular stress response pathways. Genes of interest were selected for further validation by qPCR in an independent mouse cohort.

RESULTS

1 WEEK POST IRRADIATION

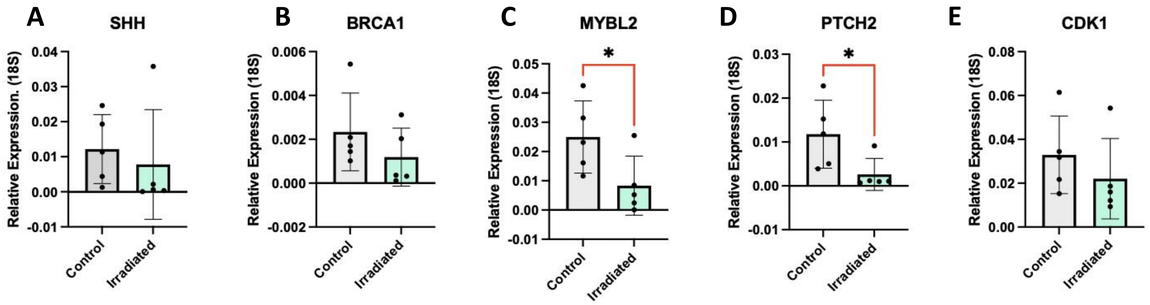


Figure 3. Downregulation of cellular stress response genes 1 week post-irradiation: (**A**) *Shh,* (**B**) *Brca1,* (**C**) *Mybl2,* (**D**) *Ptch2,* **(E)** *Cdk1.*

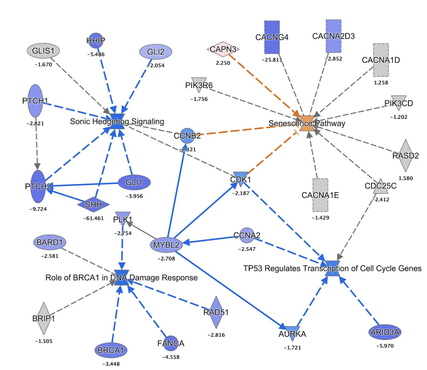


Figure 4. IPA Analysis Week 1

4 WEEKS POST IRRADIATION

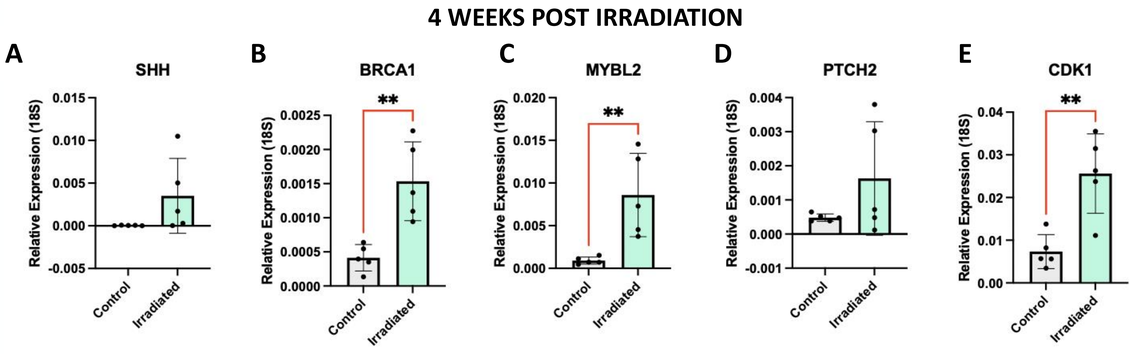


Figure 5. Gene expression changes 4 weeks post-irradiation:(**A**) *Shh,* (**B**) *Brca1*, (**C**) *Mybl2*, (**D**) *Ptch2,* **(E)** *Cdk1*

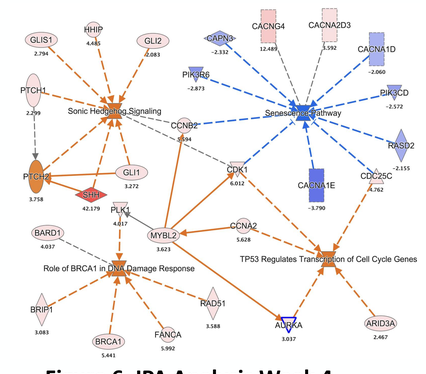


Figure 6. IPA Analysis Week 4

CONCLUSIONS AND FUTURE DIRECTIONS

Our findings indicate a **biphasic cellular stress response** to radiation. Further studies should **determine the skin structures** and **cell types** driving this transcriptomic response in the weeks following radiation.

Further understanding of the cellular stress wounding response after radiation can pave the way for **early therapeutic interventions** to mitigate cutaneous complications and **improve clinical outcomes.**

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