

Supplementary Information

Forkhead box M1 regulates quiescence-associated radioresistance of human head and neck squamous carcinoma cells

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Running title: FOXM1 and quiescent cancer cell radioresistance

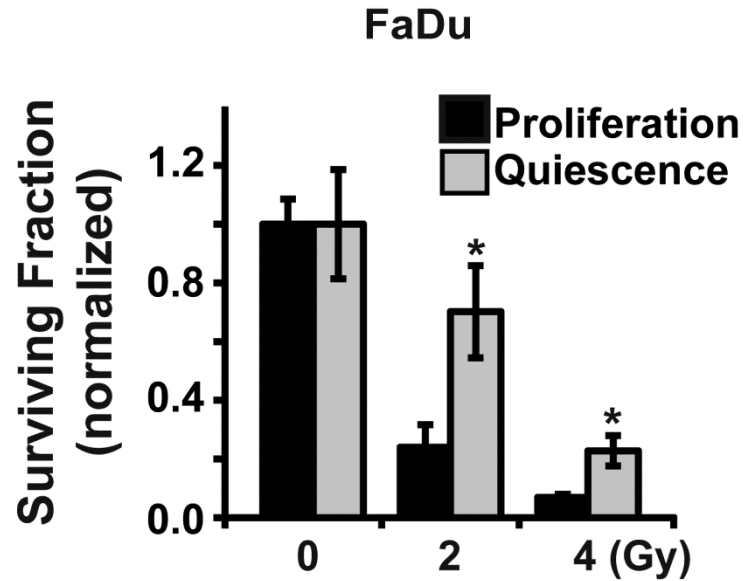
Results presented in the Supplemental section show: (1) radioresistance of quiescent HNSCC in an independent cell line, FaDu; (2) significant decrease in FOXM1 mRNA (FaDu cells) and protein levels (FaDu and Cal27) in quiescent compared to proliferating HNSCC; and (3) no perturbation in cell cycle phase distributions in FOXM1 overexpressing HNSCC.

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Supplementary Table S1**PCR primers used in this study**

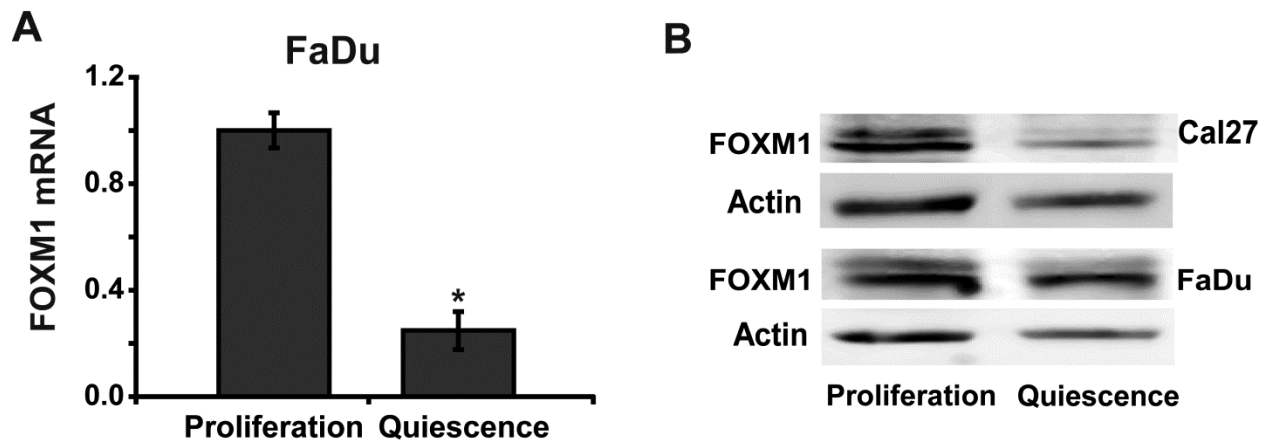
| Gene | Gene Bank No. | Sequence (5'→ 3') | Amplicon size (bp) |
|-------------|----------------------|---------------------------------|---------------------------|
| 18S rRNA | NR_077069 | Forward: CCTTGGATGTGGTAGCCGTTT | 104 |
| | | Reverse: AACTTTCGATGGTAGTCGCCG | |
| MnSOD | NM_000636.2 | Forward: TTGGCCAAGGGAGATGTTAC | 157 |
| | | Reverse: AGTCACGTTTGATGGCTTCC | |
| CAT | NM_001752.3 | Forward: CATCCAGAAGAAAGCGGTCA | 140 |
| | | Reverse: CCAAGTGAGATCCGGACTGC | |
| PRDX3 | NM_006793.2 | Forward: GCCGTTGTCAATGGAGAGTT | 179 |
| | | Reverse: CAACAGCGTCAGAGTCACCT | |
| FOXM1 | NM_202002.2 | Forward: GCAGGCTGCACTATCAACAA | 154 |
| | | Reverse: TCGAAGGCTCCTCAACCTTA | |
| DUSP1 | NM_004417 | Forward: CGCGCAAGTCTTCTTCCTCAA | 195 |
| | | Reverse: AAGGGCAGGATTTCCACCGG | |
| DUOX2 | NM_014080 | Forward: TGTGTATGAGTGGCTGCCCAGC | 113 |
| | | Reverse: ACTGCTCAGAGGCCACCACAAA | |
| CCL5 | NM_002985 | Forward: GCAGCCCTCGCTGTCATCCT | 176 |
| | | Reverse: AAGACGACTGCTGGGTTGGAGC | |
| G6PD | NM_000402.3 | Forward: CCCGGAACGGTCGTACACT | 72 |
| | | Reverse: CATGACGCTGTCTGCGCTTC | |

Supplementary Figure S1



Supplementary Figure S1: Quiescent FaDu HNSCC cells are radioresistant. A clonogenic assay designed to accommodate post-lethal damage repair was used to examine surviving fraction in FaDu HNSCC. Asterisks represent statistical significance of quiescent compared to proliferating cells. $n=3$; $P<0.05$.

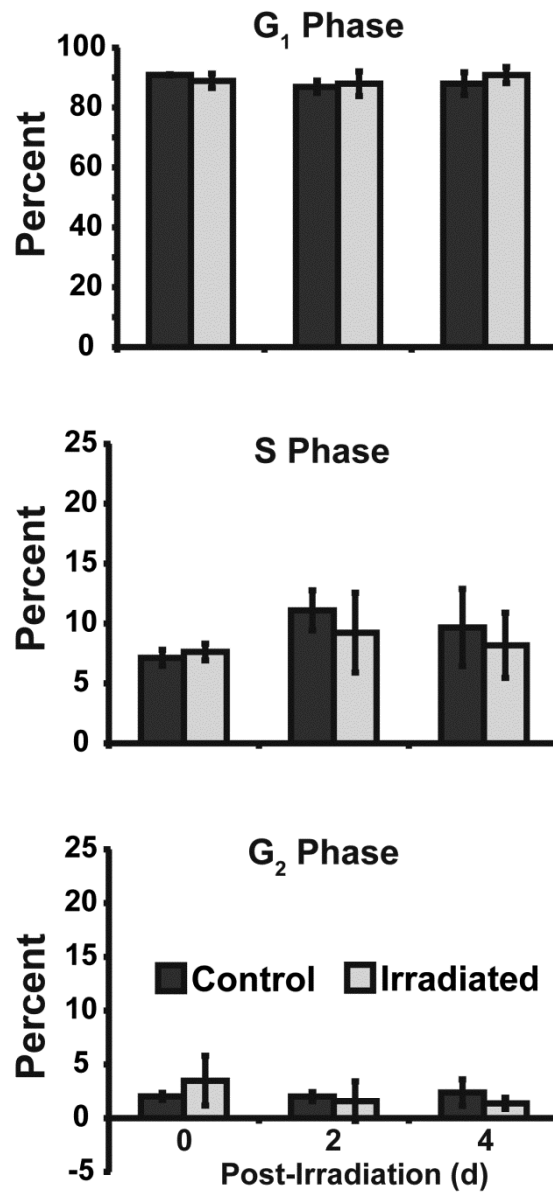
Supplementary Figure S2



Supplementary Figure S2: Quiescent HNSCC have lower expression of FOXM1

than proliferating cells. **(A)** A Q-RT-PCR assay was used to measure FOXM1 mRNA levels in proliferating and quiescent FaDu cells. Fold change in mRNA levels in quiescent cells was calculated relative to corresponding mRNA levels in proliferating cells. Asterisks represent statistical significance of quiescent compared to proliferating FaDu cells. $n=3$; $P<0.05$. **(B)** Western blot analysis of FOXM1 in proliferating and quiescent Cal27 and FaDu cells.

Supplementary Figure S3



Supplementary Figure S3: Irradiated quiescent Cal27 cells do not show a difference in cell cycle phase distribution. Flow cytometry measurements of cell cycle phase distributions in quiescent Cal27 cells following radiation.