**Role of Telomeric RAP1 in radiation sensitivity modulation and its interaction with CSC marker KLF4 in Colorectal cancer**

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**Supplementary Material**

**Supplementary Figure 1**

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Supplementary Figure 2

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Supplementary Figure 3

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Supplementary Figure 4

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Supplementary Table 1

|  |  |  |
| --- | --- | --- |
| **Genes** | **Forward Primer** | **Reverse Primer** |
| **RAP1** | 5’-ATGATGATCCACCCACACCT-3’ | 5’-AAGTAGCCTCCAGCTCACCA-3’ |
| **c-Myc** | 5’-GCTGCTTAGACGCTGGATTT-3’ | 5’-CACCGAGTCGTAGTCGAGGT-3’ |
| **β-catenin** | 5’-GCTTTCAGTTGAGCTGACCA-3’ | 5’-CAAGTCCAAGATCAGCAGTCTC-3’ |
| **KLF4** | 5’-GAAATTCGCCCGCTCCGATGA-3’ | 5’-CTGTGTGTTTGCGGTAGTGCC-3’ |
| **CD44** | 5’-CGGACACCATGGACAAGTTT-3’ | 5’-CCGTCCGAGAGATGCTGTAG-3’ |
| **OCT4** | 5’-CCTGAAGCAGAAGAGGATCA-3’ | 5’-CCGCAGCTTACACATGTTCT-3’ |
| **SOX2** | 5’-AACCCCAAGATGCACAACTC-3’ | 5’-GCTTAGCCTCGTCGATGAAC-3’ |
| **β-actin** | 5’ TCACCCACACTGTGCCCATCTACGA-3’ | 5’-CAGCGGAACCGCTCATTGCCAATGG-3’ |
| **ERCC1** | 5’ATGTGAAAGATCCCCAGCAG-3’ | 5’-GCTGGTTTCTGCTCATAGGC-3’ |

Supplementary Table 2

|  |  |
| --- | --- |
| **Protein** | 1. **Residues involved in Hydrogen Bonding** |
| **RAP1** | **Glu 310, Thr 333, Arg 392, Glu 395, Phe 396, Lys 398** |
| **KLF4** | **Lys428, Trp 434, Asp 435, Thr 448, Arg 449, His 450, Arg 452, Lys 453** |
|  | 1. **Residues involved in Hydrophobic contact** |
| **RAP1** | **Ser307, Val311, Ala314, Ile317, Ile318, Leu329, Phe336, Leu337, Ser340, Gly341, Leu343 & Arg397** |
| **KLF4** | **Trp439, Phe441, Leu447, Thr448, Tyr451, Asp433, Gly436, Thr455, Met454 & Gly456** |

\***The residues highlighted in bold and red color are shown in figures**

Supplementary Table 3

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**Supplementary Figure Legends**

**Supplementary Figure 1. a)** Graphical representation significant difference of number of colonies/500 cells post-irradiation in HCT116 and HCT116RR cells. **b)** Pictorial representation of cell survival in siRAP1 HCT116RR after IR treatment which shows a decrease in colony formation as compared to non-silenced HCT116RR cells. (*\*p <*0.05), *(\*\*p <*0.005), *(\*\*\* p <* 0.001), *(\*\*\*\* p <* 0.0001)

**Supplementary Figure 2.** **a)** Graphical representation of mRNA fold change in shelterin protein RAP1 in siRAP1 post-irradiation in HCT116, HT-29 and HCT116RR cells. **b)** KLF4 gene expression changes in silenced RAP1 cells after treatment in HCT116, HT-29 and HCT116RR cells. **c)** Down-regulation of RAP1 shows a significant decrease of β-catenin in HCT116, HT-29 and HCT116RR cells. **d)** Graphs representing a decrease in CD44 gene expression after silencing RAP1 in colorectal cancer cell lines. (\*p <0.05), (\*\*p <0.005), (\*\*\* p < 0.001)

**Supplementary Figure 3**: DNA-binding domains of KLF4 Protein. The schematic representation of all three DNA binding domain of KLF4 showing the binding site of ZnF2 domain on DNA involving Arg 443, Asp 445, Glu 446 and Arg 449. Asp445 and Glu 446 shows hydrogen bonding with methylated Cytosine.

Supplementary Figure 4: Graphical representation of local density/frustration index

Supplementary Table 1: List of primer sequences

Supplementary Table 2: Identification and positions of interface residues in docked complexes involved in the protein-protein interaction

Supplementary Table 3: Table representing the clinico-pathological features of colorectal cancer patient (n=75)