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Nrf2 facilitates repair of radiation induced DNA damage through homologous recombination repair pathway in a ROS independent manner in cancer cells

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ABSTRACT

Nrf2 is a redox sensitive transcription factor that is involved in the co-ordinated transcription of genes involved in redox homeostasis. But the role of Nrf2 in DNA repair is not investigated in detail. We have employed A549 and MCF7 cells to study the role of Nrf2 on DNA repair by inhibiting Nrf2 using all-*trans* retinoic acid (ATRA) or by knock down approach prior to radiation exposure (4 Gy). DNA damage and repair analysis was studied by γH2AX foci formation and comet assay. Results suggested that the inhibition of Nrf2 in A549 or MCF7 cells led to significant slowdown in DNA repair as compared to respective radiation controls. The persistence of residual DNA damage even in the presence of free radical scavenger *N*-acetyl cysteine, suggested that the influence of Nrf2 on DNA repair was not linked to its antioxidant functions. Further, its influence on non-homologous end joining repair pathway was studied by inhibiting both Nrf2 and DNA-PK together. This led to synergistic reduction of survival fraction, indicating that Nrf2 may not be influencing the NHEJ pathway. To investigate the role of homologous recombination repair (HR) pathway, RAD51 foci formation was monitored. There was a significant reduction in the foci formation in cells treated with ATRA or shRNA against Nrf2 as compared to their respective radiation controls. Further, Nrf2 inhibition led to significant reduction in mRNA levels of RAD51. BLAST analysis was also performed on upstream regions of DNA repair genes to identify antioxidant response element and found that many repair genes that are involved in HR pathway may be regulated by Nrf2. Together, these results suggest the involvement of Nrf2 in DNA repair, a hitherto unknown function of Nrf2, putatively through its influence on HR pathway.

1. Introduction

Radiotherapy is one of the predominant treatment modalities used in cancer treatment. But radio-resistance exhibited by the tumor cells is a major impediment in achieving the effective therapeutic outcome. Many proteins and transcription factors have been shown to determine the radio-responsiveness of the tumor cells [1]. One of the important proteins which determine the radiation response of tumor cells is nuclear factor erythroid 2-like 2 (Nrf2) [2]. Nrf2 is a redox-sensitive transcription factor, which governs the expression of many antioxidant genes and detoxifying proteins thereby maintaining the redox homeostasis of the cell. Under normal conditions, Nrf2 is sequestered in the cytoplasm by binding to an inhibitor protein kelch-like ECH-associated protein 1 (KEAP1) [3,4]. Under oxidative stress conditions, the interaction between Nrf2 and KEAP1 is disrupted leading to the translocation of Nrf2 into the nucleus resulting in transactivation of the target genes, which have antioxidant responsive elements (ARE) in their promoter regions [5,6]. Some of the known target genes of Nrf2 include many of the antioxidant enzymes and the genes which are involved in glutathione and thioredoxin antioxidant systems [5].

In many tumors, Nrf2 pathway is known to be dysregulated and thereby leading to the radioresistance during cancer therapy [7]. Several studies have demonstrated the usefulness of targeting Nrf2 for radiosensitization [8–11]. Similar to antioxidant response, DNA damage response (DDR) also is an important aspect which determines the radiation response of tumor cells. DNA damage responses include cell cycle arrest, DNA repair and apoptosis. Complex mechanisms involving many sensor proteins and effector proteins participate in DNA damage response of the cell [12]. The double strand breaks (DSB), which are considered as the most critical lesions which are responsible for cell death, are repaired by

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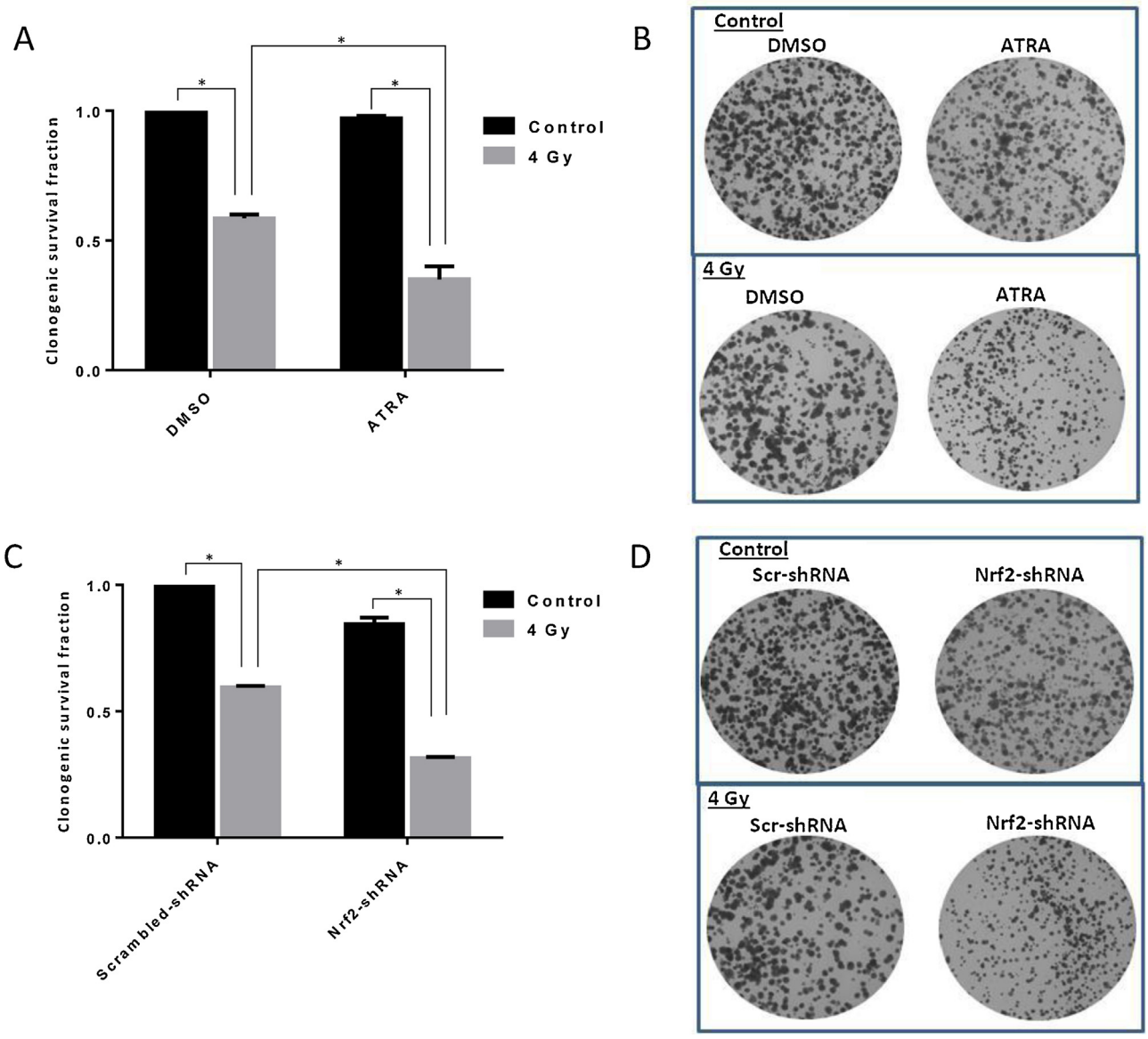

Fig. 1. Effect of Nrf2 inhibition (A and B) or Nrf2 knock down (C and D) on radiation response of A549 cells as assessed by clonogenic survival assay. For clonogenic assay, exponentially growing cells were plated overnight, treated either with ATRA 2 h prior to radiation exposure (4 Gy) or transfected with Nrf2-shRNA 24 h prior to radiation exposure. After radiation exposure, cells were allowed to grow and develop colonies for two weeks followed by fixing, staining with crystal violet, and counting of colonies using a colony counter. The representative images of the clonogenic dishes were shown (B and D). Two independent experiments were performed with triplicates each time and mean survival fraction ±SEM has been plotted. $^{*}p < 0.05$ between the two groups.

two major pathways namely non homologous end joining (NHEJ) and homologous recombination (HR) [13]. Before the repair, DNA damage is sensed by sensor proteins like ATM (Ataxia Telangiectasia Mutated), ATR, DNA-PK (DNA-dependent protein kinase) and poly ADP-ribose polymerase (PARP) proteins [14]. Binding and activation of ATM leads to the phosphorylation of many other target proteins (53BP1, H2AX, CHK2, SMC1, TP53, BRCA1, Artemis etc.), which are involved in DDR. DNA-PK is a trimeric nuclear serine/threonine kinase composed of a catalytic subunit and two DNA-targeting proteins, KU70 and KU80 [15,16]. Binding of DNA-PK initiates repair of DSB through non homologous end joining (NHEJ), which is considered as error prone repair because of the potential mis-ligation. HR repair is possible in S and G2 phase of the cell cycle if DNA resection is induced after DSB. Single strand regions of DNA generated during the DNA resection, results in binding of RAD51 protein filaments to DNA and it starts the strand invasion mediated by BRCA2 [17]. Following the strand invasion, other RAD51 family proteins encoded by RAD51B, RAD51C and RAD51D participate in late stages of HR including branch migration and Holiday junction resolution [18]. The HR results in accurate repair of the DNA due to the utilization of information from sister chromatid. Since these repair pathways are important to maintain genomic integrity of the cells, inhibition of these DNA repair pathways also has been evaluated as a potent strategy for radiosensitization by many investigators [19–21].

For exploiting the full potential of these pathways in chemo-radiotherapy of tumors, it is important to understand the interactions and crosstalk between the Nrf2 and DNA repair pathways. In this regard, our earlier study involving prostate cancer cell lines, it has been shown that the radio-resistant cell line had higher levels of Nrf2 and lower levels of DNA damage upon irradiation as compared to radio-sensitive cell line [8]. In another study, Kim et al. [22] have shown that the expression of 53BP1 (p53 binding protein) was dependent on Nrf2 in normal cells. However, stud-