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REGROWTH RESISTANCE: LOW-LEVEL PLATINUM RESISTANCE MEDIATED BY RAPID RECOVERY FROM PLATINUM-INDUCED CELL CYCLE ARREST

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Abstract

The H69CIS200 and H69OX400 cell lines are novel models of low-level platinum drug resistance developed from H69 human small cell lung cancer cells with eight 4-day treatments of 200 ng/ml cisplatin or 400 ng/ml oxaliplatin respectively. A recovery period was given between treatments to emulate the cycles of chemotherapy given in the clinic. The resistant cell lines were approximately 2-fold resistant to cisplatin and oxaliplatin and were cross resistant to both drugs. Platinum resistance was not associated with increased cellular glutathione, decreased accumulation of platinum or increased DNA repair capacity. The H69 platinum sensitive cells entered a lengthy 3 week growth arrest in response to low-level cisplatin or oxaliplatin treatment. This is an example of the coordinated response between the cell cycle and DNA repair. In contrast the H69CIS200 and H69OX400 cells have an alteration in the cell cycle allowing them to rapidly proliferate post drug treatment. The resistant cell lines also have many chromosomal rearrangements most of which are not associated with the resistant phenotype, suggesting an increase in genomic instability in the resistant cell lines. We hypothesised that there was a lack of coordination between the

cell cycle and DNA repair in the resistant cell lines allowing proliferation in the presence of DNA damage which has created an increase in genomic instability. The H69 cells and resistant cell lines have mutant p53 and consequently decrease the expression of p21 in response to platinum drug treatment, promoting progression of the cell cycle instead of increasing p21 to maintain the arrest. A decrease in ERCC1 protein expression and an increase in RAD51B foci activity was observed with the platinum induced cell cycle arrest and did not correlate with resistance or altered DNA repair capacity. These changes may in part be mediating and maintaining the cell cycle arrest in place of p21.The rapidly proliferating resistant cells have restored the levels of both these proteins to their levels in untreated cells. We use the term 'regrowth resistance' to describe this low-level platinum resistance where cells survive treatment through increased proliferation. Regrowth resistance may play a role in the onset of clinical resistance.

Chapter

The H69CIS200 cisplatin-resistant and H69OX400 oxaliplatin-resistant small cell lung cancer cell lines are novel models of low-level platinum resistance (1). The H69CIS200 and H69OX400 cell lines were developed from parental H69 small cell lung cancer cells with eight 4-day treatments of 200 ng/ml cisplatin or 400 ng/ml oxaliplatin respectively with a recovery period between treatments to emulate the cycles of chemotherapy given in the clinic. These cell lines are approximately 2-fold resistant to cisplatin and oxaliplatin and are cross resistant to both drugs. The resistance is not associated with increased cellular glutathione or decreased accumulation of platinum which are common mechanisms of platinum resistance. The H69 platinum sensitive cells enter a lengthy 3 week growth arrest in response to low-level cisplatin and oxaliplatin treatment (Fig. 1A). This is an example of the coordinated response between the cell cycle and DNA repair. In contrast the H69CIS200 and H69OX400 cells have an alteration in the cell cycle allowing them to rapidly proliferate post drug treatment (Fig. 1B). We use the term 'regrowth resistance' to describe this low-level platinum resistance where cells survive treatment through increased proliferation. Regrowth resistance may play a role in the onset of clinical resistance.

The resistant cell lines also have many chromosomal rearrangements most of which are not associated with the resistant phenotype, suggesting an increase in genomic instability in the resistant cell lines (2). The H69 cells and resistant cell lines have mutant p53 and consequently decrease the expression of p21 in response to platinum drug treatment, promoting progression of the cell cycle instead of increasing p21 to maintain the arrest (data not shown). We hypothesised that there was a lack of

coordination between the cell cycle and DNA repair in the resistant cell lines allowing proliferation in the presence of DNA damage which has created an increase in genomic instability. Increased DNA repair is a common mechanism of platinum resistance. However, the H69CIS200 and H69OX400 cells showed no change in DNA repair capacity as measured by a repair of a platinated plasmid and expression of DNA repair marker γH2AX (data not shown).

Despite no change in DNA repair capacity in the resistant cell lines we found alterations in the expression and activity of two DNA repair proteins. ERCC1 is involved in the nucleotide excision repair removal of platinum adducts from DNA, increased ERCC1 mRNA and protein expression has been associated with cisplatin resistance (3). In contrast, we observed decreases in ERCC1 protein expression (Fig. 2A), which were associated with the formation of a lower molecular weight band of approximately 26 kDa (marked with arrow Fig. 2A). We believe this to be an alternative spliced variant of ERCC1 associated with decreased repair activity (4). The samples in cell cycle arrest (grey background) had a significant decrease in ERCC1 protein expression compared to the untreated control cells (Fig. 2B). This suggests that ERCC1 expression is more related to cell cycle state than resistance. The samples in recovering from cell cycle arrest were not significantly different from the untreated control cells but had lower levels of mRNA and protein suggesting that part of restoring normal cell cycle activity was associated with restoring normal ERCC1 levels.

Homologous recombination repair is mediated in part by the RAD51 proteins (5). An increase in homologous recombination could mediate platinum resistance by

increasing the repair of platinum induced double-strand DNA breaks. We chose to examine RAD51B as it has been linked to both cell cycle control and DNA repair (6). We observed no change in RAD51B protein expression (data not shown). However, activity as measured by the presence of nuclear RAD51 foci did change. RAD51B foci were examined by immunocytochemistry in the H69, H69CIS200 and H69OX400 cell lines (Fig. 2C). The parental H69 cells had higher levels of RAD51B foci in response to oxaliplatin drug treatment than the H69OX400 cells. This is the opposite of what would be expected, since the resistant cells would be expected to have a higher level of repair than the sensitive parental cells. RAD51B activity was increased significantly in the arrested cells compared to the non-arrested controls, suggesting that its expression and activity are related more to the cell cycle than to platinum resistance. The samples in cell cycle recovery had no change in RAD51B foci from the untreated cells suggesting that part of restoring normal cell cycle activity was restoring normal RAD51B foci activity.

The changes in the ERCC1 and RAD51B are associated with cell cycle arrest rather than resistance, suggesting that they are being modulated for reasons other than DNA repair and are potentially participating in the regrowth resistance mechanism of cell cycle arrest and recovery. There is some evidence to suggest that ERCC1 and RAD51B could mediate a cell cycle arrest. Hepatocytes from ERCC1 knockout mice are arrested in the G_2 phase of the cell cycle (8). The expression of full length ERCC1 decreases in association with the cell cycle arrest, however this is associated with the formation of an ERCC1 splice variant which has been previously reported to have reduced DNA repair activity (4). It is possible that this splice variant may have an increased role in the process of cell cycle arrest. Fibroblasts from ERCC1 knockout

mice also show a decreased rate of cell growth and disruptions in cell cycle (9) suggesting that the decrease in ERCC1 may contribute to the lengthy growth arrest in the sensitive cells. Transfection of RAD51B into CHO cells induces a cell cycle G_1 delay similar to what was observed in the H69 cells in response to platinum treatment (10). Transfection of RAD51 into human and rat fibroblasts also induces a G_1 arrest (7).

Resistance in the H69CIS200 and H69OX400 cells is dependent on a rapid cell cycle progression after drug treatment (Fig. 1B). The regrowth resistance arrest is the same in all cells, however, the resistant cell lines quickly exit this cell cycle arrest and continue to cycle despite the presence of DNA damage. Therefore the resistant cells have a decrease in DNA repair in response to platinum drug treatment, not because of a downregulation of a DNA repair pathway but because of the reduced time in cell cycle arrest where the repair occurs. Decreases in ERCC1 (11) and increases in RAD51 (12) have also been associated with increased genomic instability which correlate with the large amount of chromosomal aberrations found in the resistant cell lines (2).

The normal exist from the cell cycle arrest after the successful completion of DNA repair is termed checkpoint recovery. Normal checkpoint recovery in the H69 parental cells is the 3 week growth arrest (Fig. 1A). Checkpoint adaptation is related to checkpoint recovery and promotes cell cycle re-entry even when unrepairable DNA damage is present (13). The H69CIS200 and H69OX400 cells appear to have the checkpoint adaptation phenotype, the cell cycle continuing despite the presence of DNA damage. The H69OX400 cells exit the cell cycle arrest faster than the

H69CIS200 cells and this correlates with the greater amount of chromosomal aberrations in the H69OX400 cell line (2).

Conclusions

Resistance in the H69CIS200 and H69OX400 cells is associated with the speed of the recovery from the cell cycle arrest, termed regrowth resistance, which may involve modulation of ERCC1 and RAD51B.

References

(1) Stordal BK, Davey MW, Davey RA. Oxaliplatin induces drug resistance more rapidly than cisplatin in H69 small cell lung cancer cells. Cancer Chemotherapy & Pharmacology 2006; 58(2):256-265.

(2) Stordal B, Peters G, Davey R. Similar chromosomal changes in cisplatin and oxaliplatin-resistant sublines of the H69 SCLC cell line are not associated with platinum resistance. Genes, Chromosomes & Cancer 2006; 45(12):1094-1105.

(3) Altaha R, Liang X, Yu JJ, Reed E. Excision repair cross complementing-group 1: gene expression and platinum resistance. International Journal of Molecular Medicine 2004; 14(6):959-970.

(4) Yu JJ, Mu C, Dabholkar M, Guo Y, Bostick-Bruton F, Reed E. Alternative splicing of ERCC1 and cisplatin-DNA adduct repair in human tumor cell lines. International Journal of Molecular Medicine 1998; 1(3):617-620.

(5) Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annual Review of Biochemistry 2004; 73:39-85.

(6) Havre PA, Rice M, Ramos R, Kmiec EB. HsRec2/Rad51L1, a protein influencing cell cycle progression, has protein kinase activity. Experimental Cell Research 2000; 254(1):33-44.

(7) Raderschall E, Bazarov A, Cao J, Lurz R, Smith A, Mann W et al. Formation of higher-order nuclear Rad51 structures is functionally linked to p21 expression and protection from DNA damage-induced apoptosis. Journal of Cell Science 2002; 115(Pt 1):153-164.

(8) Nunez F, Chipchase MD, Clarke AR, Melton DW. Nucleotide excision repair gene (ERCC1) deficiency causes G2 arrest in hepatocytes and a reduction in liver binucleation: the role of p53 and p21. FASEB J 2000; 14(9):1073-1082.

(9) Weeda G, Donker I, de Wit J, Morreau H, Janssens R, Vissers CJ et al. Disruption of mouse ERCC1 results in a novel repair syndrome with growth failure, nuclear abnormalities and senescence. Current Biology 1997; 7(6):427-439.

(10) Havre PA, Rice MC, Noe M, Kmiec EB. The human REC2/RAD51B gene acts as a DNA damage sensor by inducing G1 delay and hypersensitivity to ultraviolet irradiation. Cancer Research 1998; 58(20):4733-4739.

(11) Sargent RG, Meservy JL, Perkins BD, Kilburn AE, Intody Z, Adair GM et al. Role of the nucleotide excision repair gene ERCC1 in formation of recombination-dependent rearrangements in mammalian cells. Nucleic Acids Research 2000; 28(19):3771-3778.

(12) Richardson C, Stark JM, Ommundsen M, Jasin M. Rad51 overexpression promotes alternative double-strand break repair pathways and genome instability. Oncogene 2004; 23(2):546-553.

(13) Harrison JC, Haber JE. Surviving the breakup: the DNA damage checkpoint. Annual Review of Genetics 2006; 40:209-235.

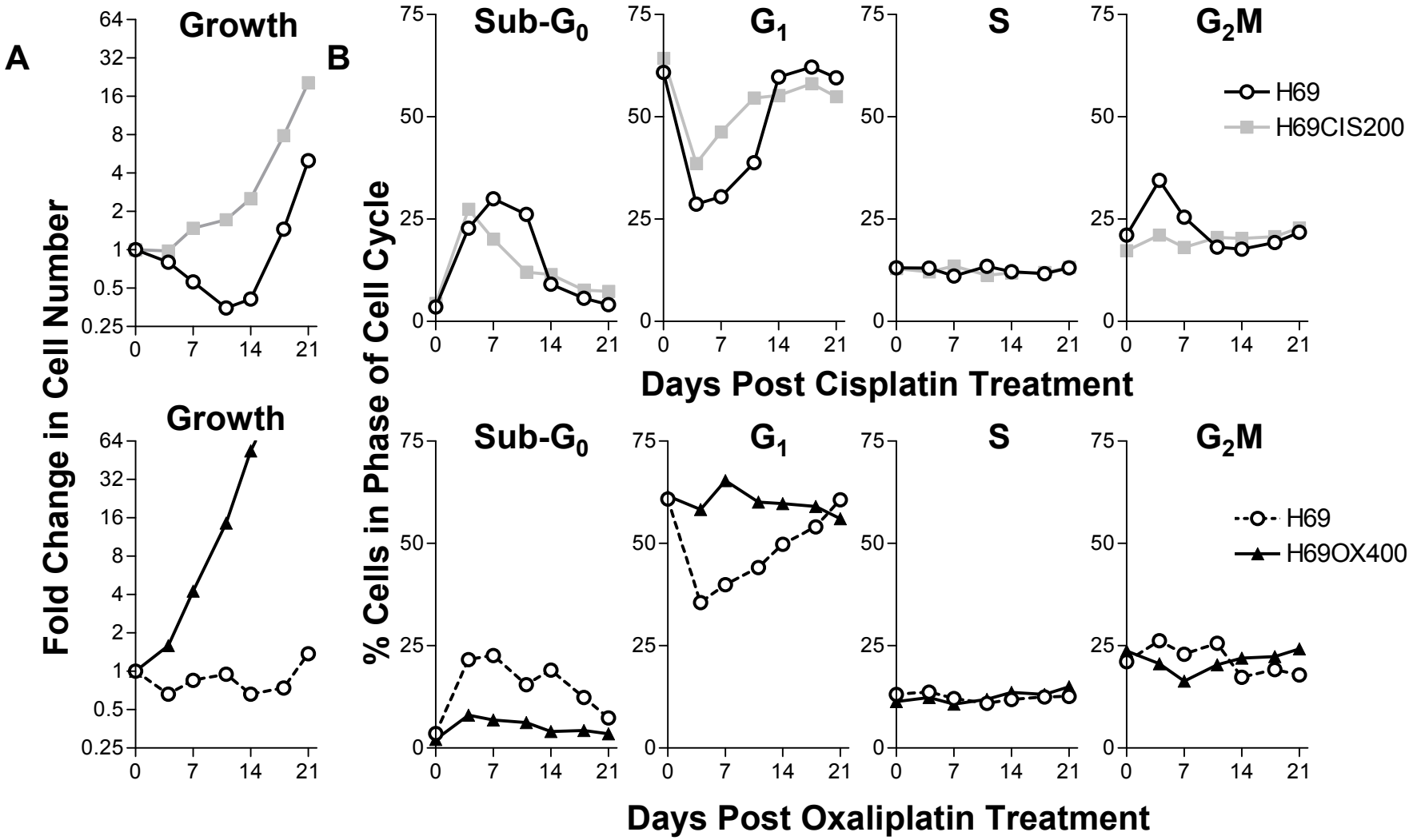

Fig. 1. Effect of acute drug treatment on cell growth and cell cycle. Cells were treated with either 1000ng/ml cisplatin or 2000 ng/ml oxaliplatin for 2 hours as indicated. A) The number of cells that exclude trypan blue were counted and the fold change in growth was plotted. B) The proportion of cells in each phase of the cell cycle was determined by the propidium iodide/flow cytometry.

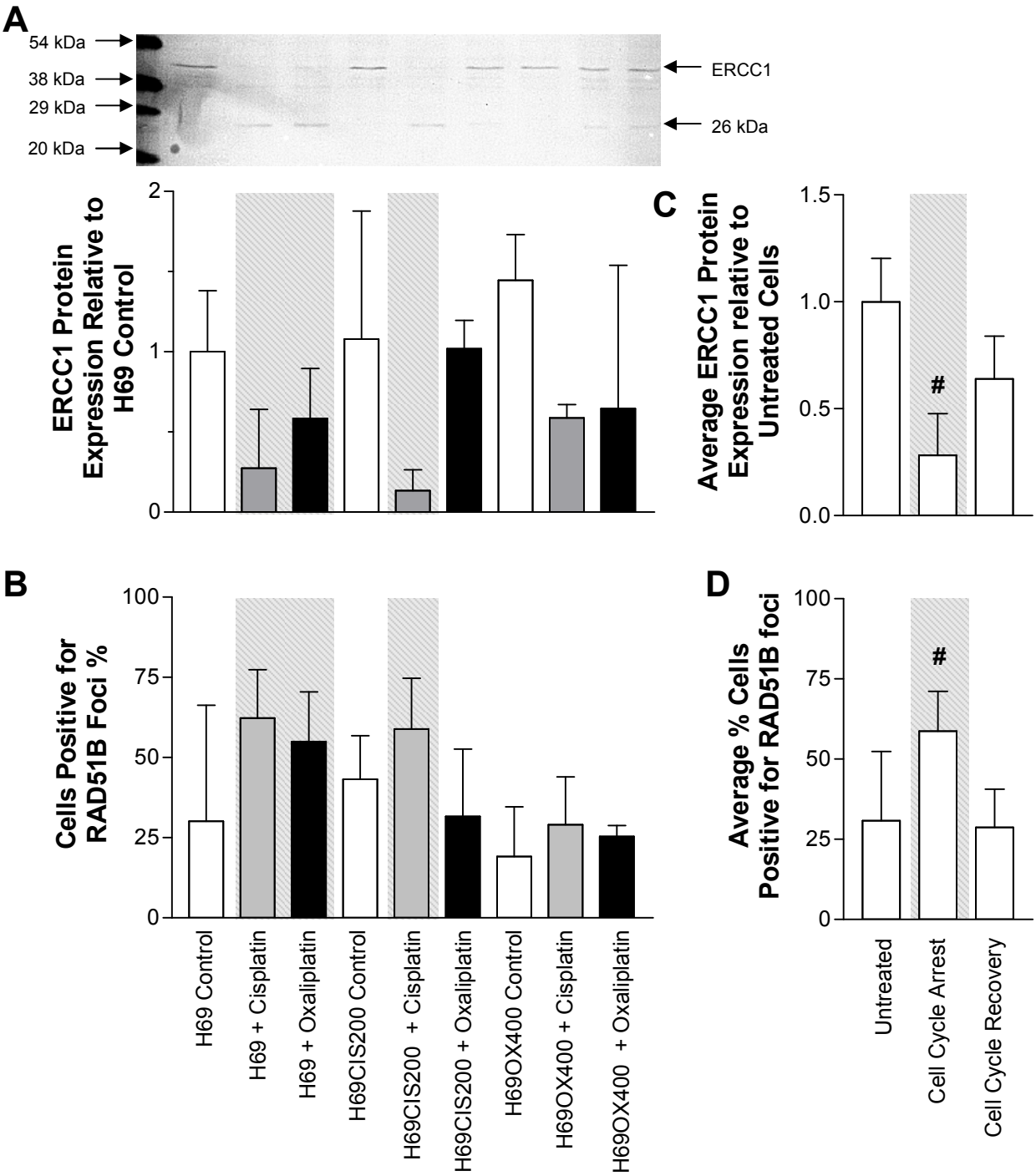

Fig. 2– Analysis of ERCC1 protein expression and RAD51B activity in H69, H69CIS200 and H69OX400 cells after a 4-day exposure either 200 ng/ml cisplatin or 400 ng/ml oxaliplatin. Samples in cell cycle arrest are indicated with a gray background. A) ERCC1 protein expression determined by Western Blot and B) analysis in reference to the cell cycle. C) RAD51B activity determined by immunocytochemistry and D) analysis in reference to the cell cycle. Means and standard deviations are presented from pooled data from parts A and C. Untreated is the control cells, cell cycle arrest is the drug treated samples in cell arrest indicated with gray background shading, cell cycle recovery is the drug treated cells not in cell cycle arrest. # indicates a significant difference in compared to the untreated samples.