Increased replication stress sensitises high risk neuroblastoma cells to ATR and PARP inhibition

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Background

- Neuroblastoma (NB) is a rare childhood cancer, half of which are high risk and a survival rate of 50%
- *MYCN* amplification and/or *ATM* loss through 11q deletion are common features of high-risk NB and may increase replication stress (RS).
- RS is a state in which the DNA replication machinery cannot maintain the rate of DNA synthesis resulting in increased replication fork stalling and collapse, chromosome instability and ultimately cell death.
- **Cells with high levels of RS are acutely dependent on the ATR signalling pathway for survival.**

Aims

1. To determine if *MYCN* amplification or ATM loss identifies cells which are sensitive to ATR inhibition (ATRi).
2. To examined the effect of ATRi on PARP inhibitor (PARPi) cytotoxicity and PARPi-induced RS, cell cycle arrest and HRR activity.

Results

1. NB cell lines with high MYCN or low ATM protein expression have increased sensitivity to ATR inhibition

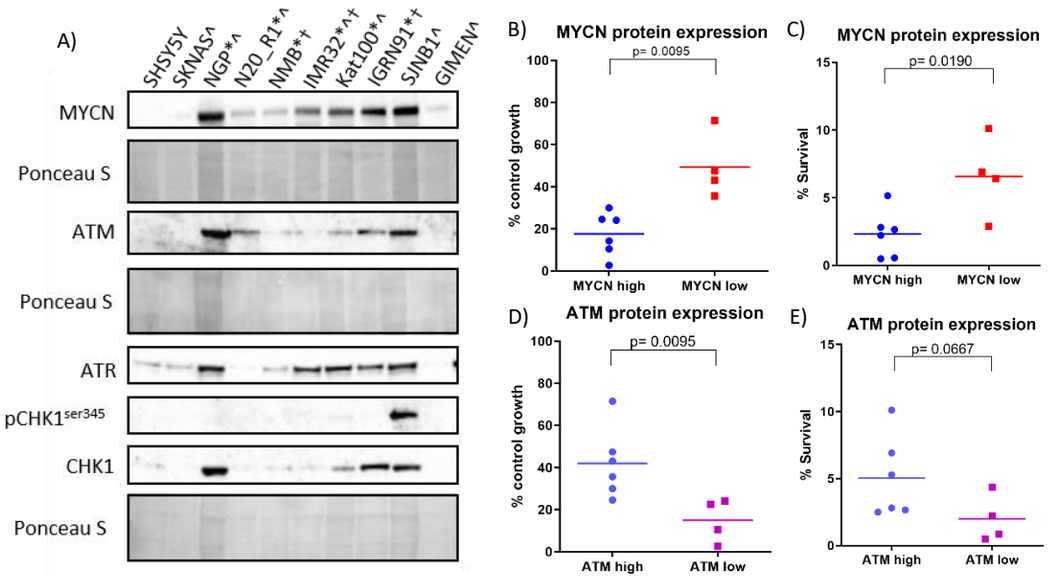

Figure 1: A) Representative Western blot images of baseline expression of MYCN, ATM, ATR, phospho-CHK1^{S345} (marker of ATR activity) and CHK1 in 10 NB cell lines. **MYCN* amplified, ^11q deleted, † *ATM* mutant. Ponceau S stain was used as measure of total protein loading for control. Cell lines were split into 2 groups based on MYCN (B and C) or ATM (D and E) protein expression (Western blot). Average percentage control growth (XTT cell proliferation) and clonogenic survival at 10 µM VE-821 was plotted for cell lines belonging to each group (n=3).

2. ATR inhibition potentiates PARPi-induced growth inhibition and RS

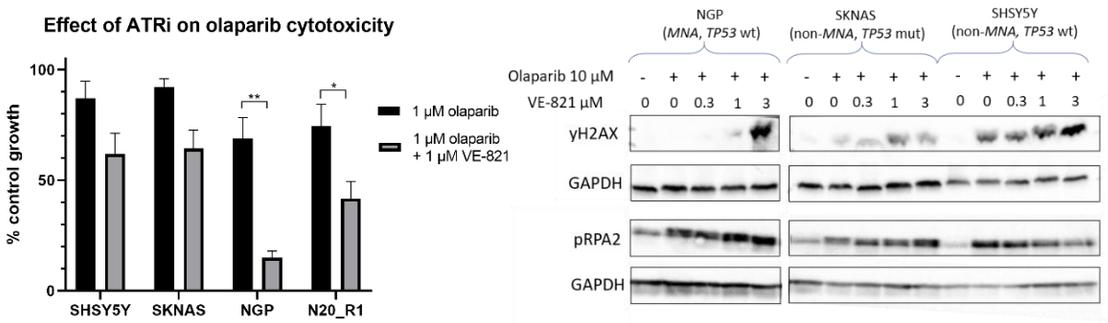

Figure 2: A) Effect of 1 µM VE-821 on cytotoxicity of 1 µM olaparib normalised to the effect of VE-821 alone. Data shown are the mean + SEM from 4 individual experiments. 2-way ANOVA: *p<0.05, ** p<0.01, C) Combination index (CI) values were calculated using CalcuSyn and plotted in B) Western blot analysis of γH2AX and phospho-RPA^{S8} (pRPA2) in the NGP, SKNAS and SHSY5Y cell lines after treatment with 10 µM olaparib with and without 0.3, 1 or 3 µM VE-821 for 24 hours. GAPDH was used as a control for protein loading.

Results

2. ATR inhibition potentiates PARPi-induced growth inhibition and RS (continued)

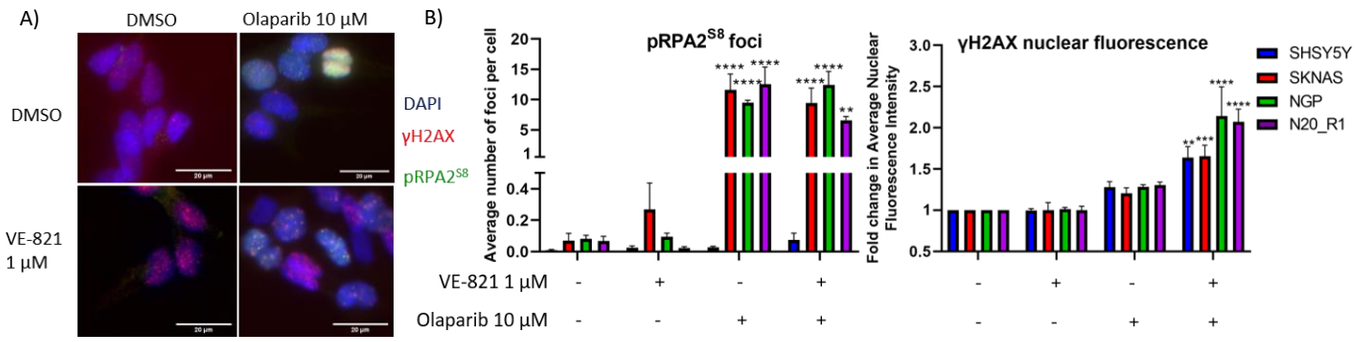

Figure 3: A) Representative γH2AX and pRPA2^{S8}foci images from the NGP cell line treated with 1 µM VE-821, 10 µM olaparib or both for 24 hours. B) Average number of pRPA2^{S8} and fold change in mean γH2AX total nuclear fluorescence intensity for SHSY5Y, SKNAS, NGP and N20_R1 cell lines treated as in A. Data are mean + SEM from 3 independent experiments. ** p<0.01, *** p<0.001, **** p<0.0001, 2-way ANOVA difference from control (DMSO).

3. ATR inhibition reduces PARPi-induced HRR foci

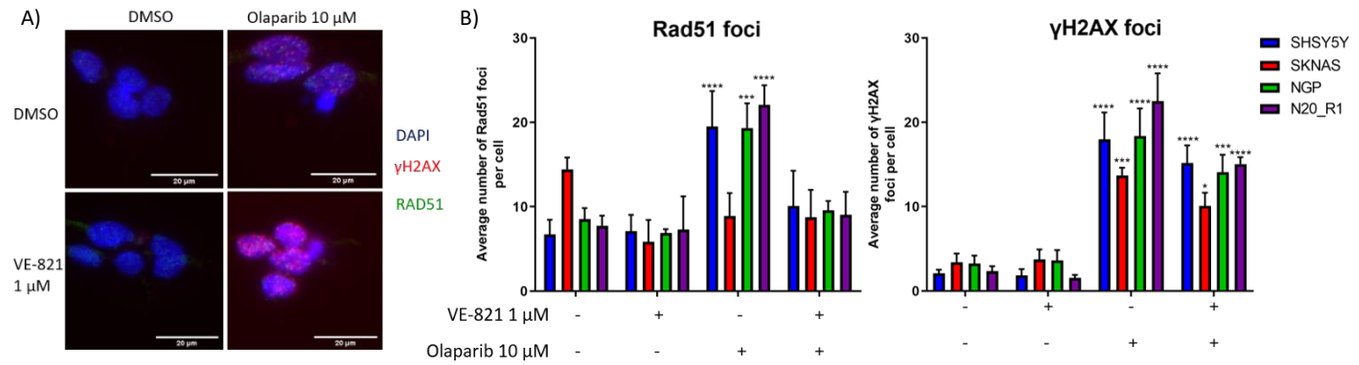

Figure 4: A) Representative γH2AX and Rad51 foci images from the NGP cell line treated with 1 µM VE-821, 10 µM olaparib or both for 24 hours. B) Average number of Rad51 and γH2AX foci per cell for SHSY5Y, SKNAS, NGP and N20_R1 cell lines treated as in A. Data are mean + SEM from 4 independent experiments. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, 2-way ANOVA difference from control (DMSO).

4. ATR inhibition abrogates PARPi-induced S and G2 arrest

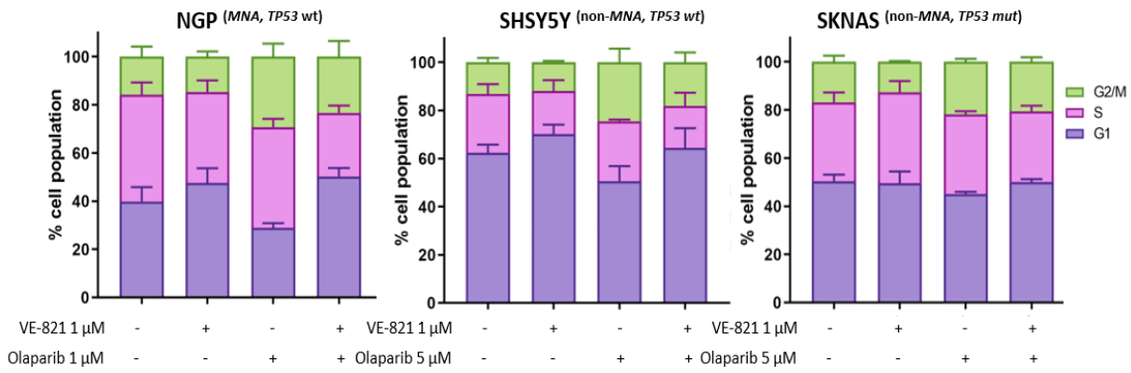

Figure 5: Cell cycle phase distribution of SHSY5Y, SKNAS and NGP cell lines treated with 5 µM (SHSY5Y and SKNAS) or 1 µM (NGP) olaparib in combination with 1 µM VE-821 for 24 h. Data are mean + SEM from 3 independent experiments. *MNA: MYCN* amplified, wt: wild type, mut: mutant

Conclusions

1. MYCN overexpression and low ATM protein expression are determinants of ATRi sensitivity in NB cell lines.
2. ATRi sensitises NB cells to PARPi by abrogating S/G2 checkpoint arrest and impairing HRR.