

G-quadruplexes and DNA damage in colorectal cancer tumorigenesis

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INTRODUCTION

- Non-canonical four-stranded G-quadruplex (G4) structures form through self-recognition of guanines into stacked tetrads within chromatin DNA.
- Considerable evidences exist for G4s formation *in vitro* and have linked G4 formation with key biological processes ranging from transcription and translation to genome instability and cancer.
- G4s have not been exploited for colorectal cancer (CRC) treatment.

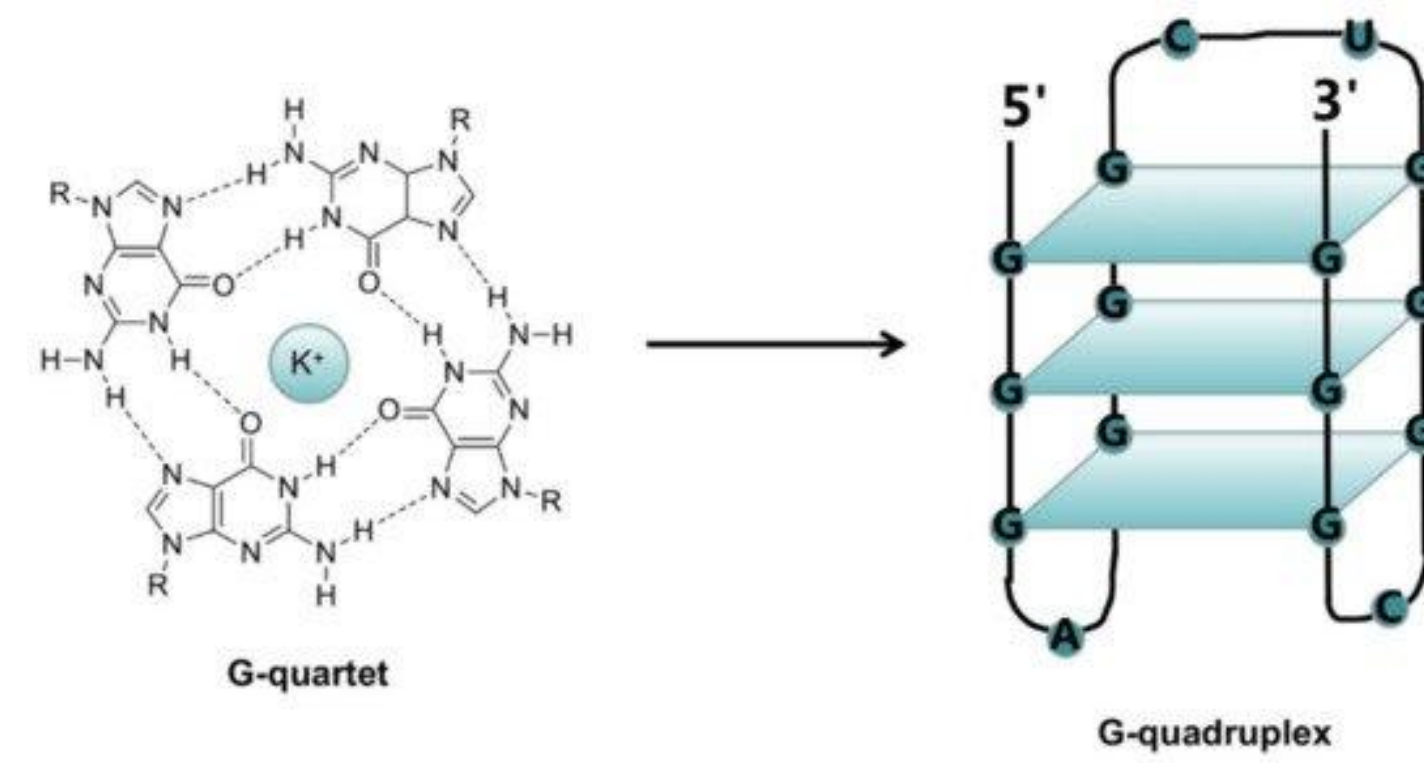


Figure 1. G4 structure.

OBJECTIVES

- To assess G4s as therapeutic targets in CRC tumorigenesis.
- To characterize the role of G4-mediated genomic instability in CRC.

WORKFLOW

1. Establishment of a CRC cellular model

- CRL1790: Colon epithelial cells
- SW480: Colorectal adenocarcinoma

2. Cell cycle arrest

- G0/G1: serum deprivation 48h
- S: thymidine 1.5 mM 48h

3. Quantification of G4 and DNA damage levels

- BG4 and gH2AX Immunofluorescence
- Fiji quantification (N > 200)

4. Investigation of relationship between G4s and DNA damage

- BG4 and gH2AX colocalization analysis

5. Evaluation of G4s as therapeutic targets

- Resazurin method at 48h upon treatment with G4s-ligands

RESULTS

Figure 2. G4s abundance increases in CRC. Images showing BG4 foci (green) in CRL1790 and SW480 cell nuclei (blue) to detect G4s abundance at G0/G1 and S phases. Respective Fiji quantification is shown in the right panel.

Figure 3. DNA damage increases in CRC. Images showing gH2AX foci (red) in CRL1790 and SW480 cell nuclei (blue) to detect gH2AX abundance at G0/G1 and S phases. Respective Fiji quantification is shown in the right panel.

Figure 4. DNA damage is only partially linked to G4s presence. Images of cell nuclei (blue) co-stained for BG4 (green) and gH2AX (red) in SW480 cells arrested at S phase.

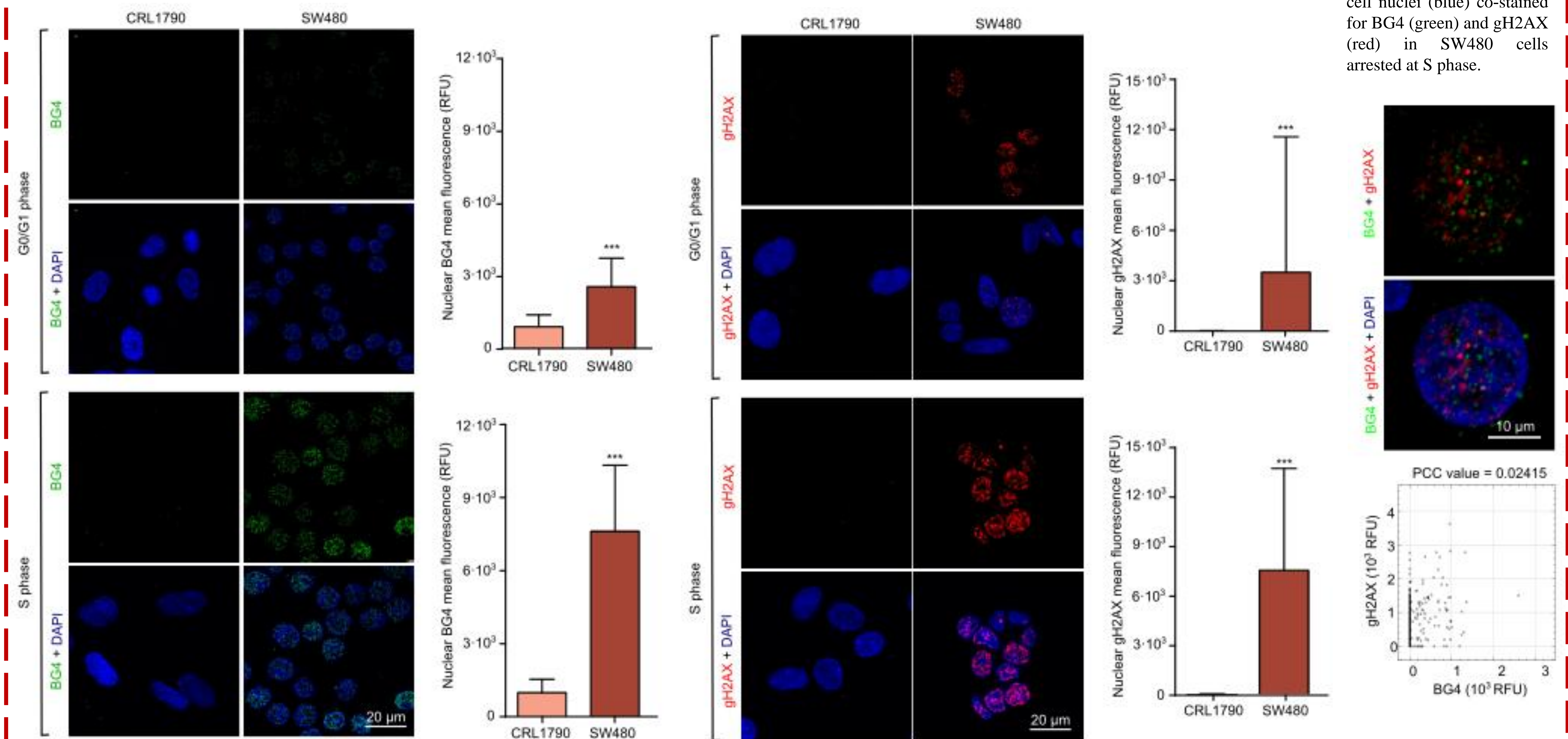
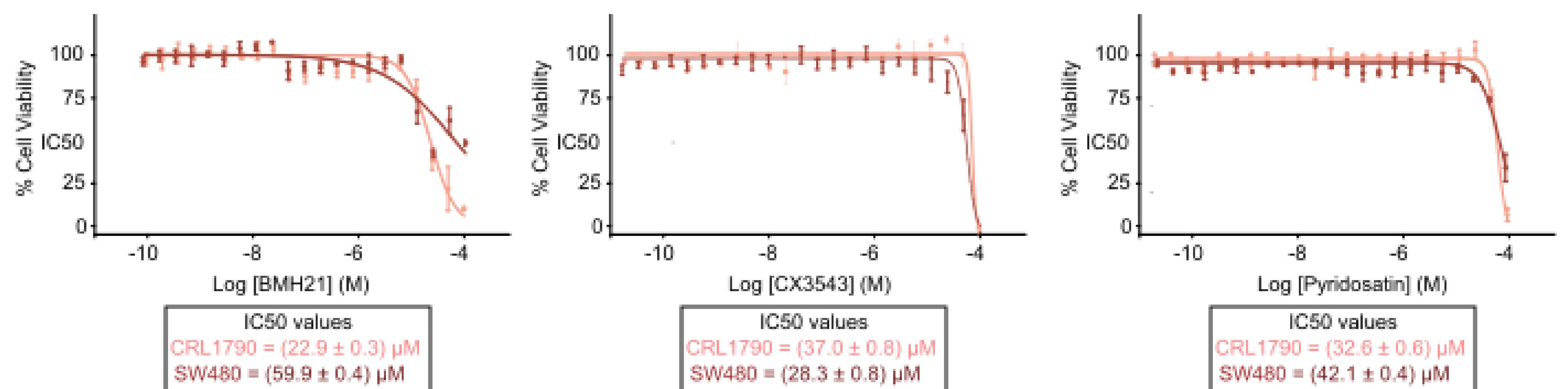


Figure 5. IC50 values for G4-ligands in CRC. IC50 values represent G4-ligand concentration inhibiting cell growth by 50% and are expressed as mean \pm standard deviation.



CONCLUSIONS

- Non-tumoral epithelial cells showed the lowest G4 and gH2AX levels, which significantly increased in CRC.
- Genome instability in CRC was only partially explained by G4s presence.
- G4-ligands showed cytotoxic activity in CRC but lacked tumoral selectivity.