Inverse relationship between G-quadruplex and i-motif structures in EMT

Jessica. J. King, Cameron. W. Evans, Sugandha. Bhatia, Erik. W. Thompson, K. Swaminathan Iyer, Nicole. M. Smith*

School of Molecular Sciences, The University of Western Australia
Perth, WA, Australia and Invasion and Metastasis Unit, Queensland University of Technology, Brisbane, QLD, Australia
jessica.king@research.uwa.edu.au

Certain guanine- and cytosine-rich sequences can assemble into non-canonical secondary DNA structures; G-quadruplex and i-motif. G-quadruplexes and i-motifs are upregulated in cancer cells and are highly enriched in promoter sequences, where they regulate protein binding and transcription. These structures are highly cell cycle dependent, with i-motifs most prominent during G1 phase (high levels of transcription)[1] and G-quadruplexes most abundant during S phase (DNA replication)[2]. We have previously shown that G-quadruplexes and i-motifs have an interdependent relationship which suggests that these structures do not form concomitantly[3].

G-quadruplex structures in the ZEB1[4] and SNAI1[5] promoter have been investigated using biophysical techniques and display a role in silencing transcription. However, these structures and their relationship with EMT remains largely unknown. This study aimed to investigate G-quadruplex and i-motif formation throughout EMT, using the HMLE cell line and the PMC42-LA breast cancer cell model. The structure specific scFv antibodies BG4[2] and iMab[1] were used for immunofluorescence to visualise and quantify G-quadruplex and i-motif structures respectively, throughout EMT progression. The results indicate that G-quadruplex structures are more prominent in epithelial cells where as the number of i-motif structures increases once EMT has been initiated and peaks in stable mesenchymal cells. This suggests that G-quadruplex and i-motif structures may be used as alternative markers for EMT cell state.

G-quadruplex and i-motif structures can be stabilised via small compounds that recognise and bind to the target structure. Pyridostatin is a well-known compound that selectively binds and stabilises G-quadruplex structures.[2] HMLE cells were treated with pyridostatin and EMT progression over a 12 d period was monitored utilising immunofluorescence for E-cadherin and vimentin proteins (epithelial and mesenchymal markers respectively). When cells were exposed to TGF-β1, a commonly used EMT trigger, pyridostatin treatment slowed down vimentin protein expression and maintained high E-cadherin levels, indicating that these structures may be targeted to regulate EMT progression.

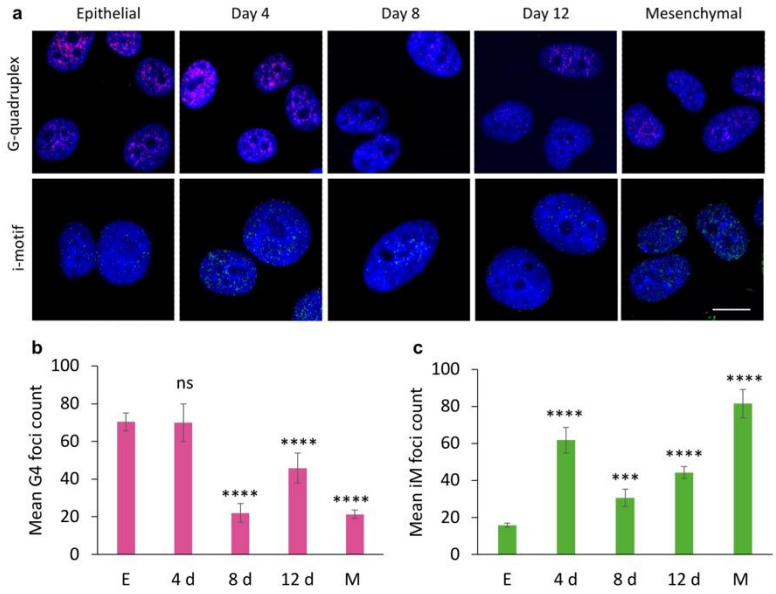

Figure 1: Visualisation and quantification of G-quadruplex and i-motif structures throughout TGF-β triggered EMT in the HMLE cell line. (a) Representative images of G-quadruplex (red) and i-motif (green) foci in HMLE nuclei (blue) at different EMT timepoints. (b-c) Quantification of G-quadruplex (b) and i-motif (c) foci in epithelial (E), mesenchymal (M) and after 4, 8, and 12 days of TGF-β treatment (2 ng/mL). 65-104 nuclei were counted per sample, with mean and SEM displayed. Statistical significance shown relative to E. p***< 0.0001, p****<0.00001, ns = non-significant.

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