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Krishna Raut
Old Dominion University

Samjhana Pandey
Old Dominion University

Andrea M. Clark
Old Dominion University

Komala Ponniah
Old Dominion University

Steven M. Pascal
Old Dominion University

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Krishna K. Raut[1], Samjhana Pandey[2], Andrea M. Clark[1], Komala Ponniah[1], and Steven M. Pascal[1]

1 Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA 23508, United States

2 Biomedical Sciences, Old Dominion University, Norfolk, VA 23529, United States

Email: kraut001@odu.edu

INTRODUCTION

Prostate apoptosis response-4 (Par-4) is a pro-apoptotic tumor suppressor protein. Down-regulation of this protein has been reported in a myriad of cancers whereas up-regulation is associated with several neurodegenerative disorders. Par-4 is unique in the sense it can selectively induce apoptosis in cancer cells. For this, caspase-dependent intracellular cleavage of Par-4 is essential to produce the functionally active fragment, cl-Par-4 (caspase-cleaved Par-4). Our laboratory aims to characterize the structure of cl-Par-4 *in vitro*.

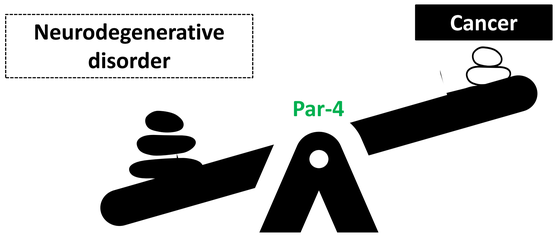

Fig. 1 Schematic showing results of Par-4 imbalance.

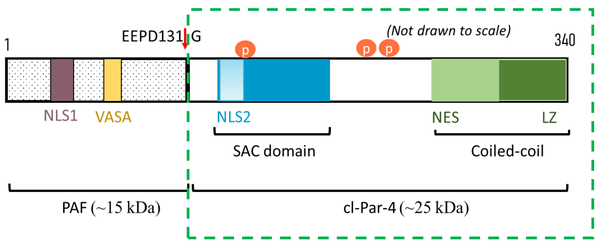

Fig. 2 Schematic showing domains of Par-4.

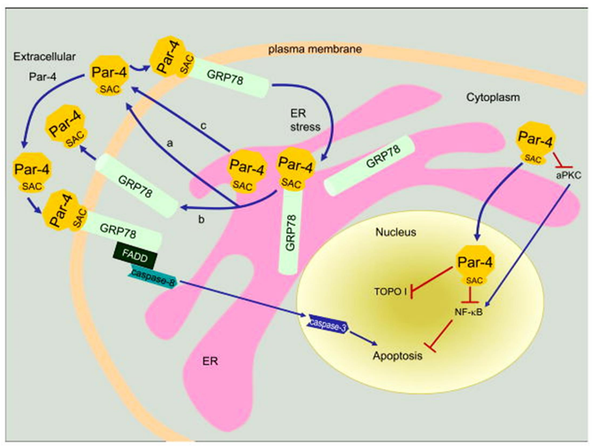

Fig. 3 Schematic showing apoptosis induction by Par-4 in a cancer cell.

RESULTS

Intrinsic disorder prediction in cl-Par-4

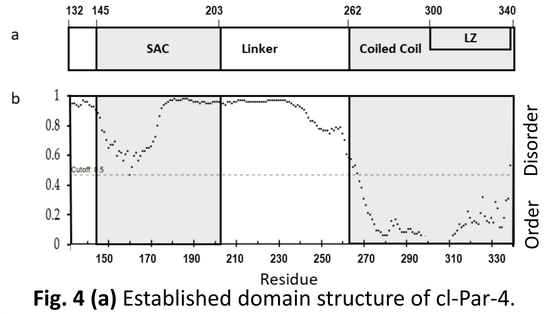

Fig. 4 (a) Established domain structure of cl-Par-4. **(b)** Disorder prediction using DISOPRED3.

Divalent cation exerts similar effect as higher concentration of monovalent cations

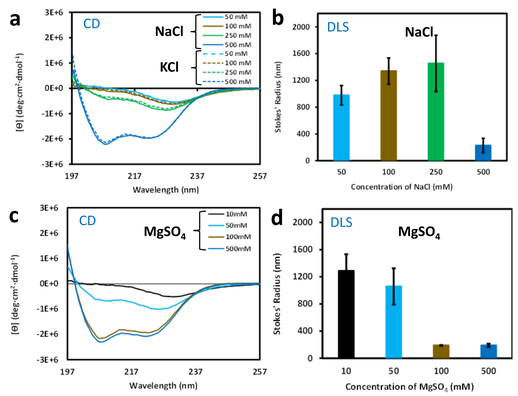

Fig. 5 CD spectra (a) & (c) and DLS (b) & (d) of cl-Par-4 in presence of various concentration of salts at pH 7.

Low pH exerts similar effect as higher concentration of cations at neutral pH on cl-Par-4

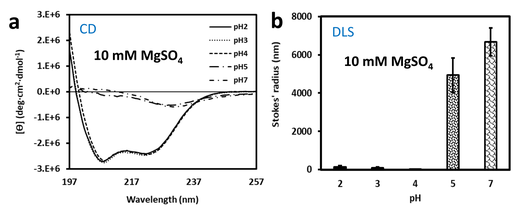

Fig. 6 CD spectra and DLS of cl-Par-4 in presence of 10 mM $MgSO_4$ salt at different pH.

Repulsion between charged residues causes Par-4 aggregation

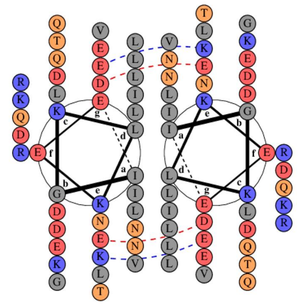

Fig. 7 Helical wheel representation of Par-4 leucine zipper parallel dimer (red-dashed line: inter-helical charge repulsion; blue-dashed line: inter-helical salt bridge formation).

D313K mutant of cl-Par-4 requires less salt

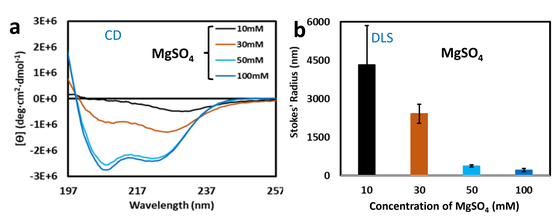

Fig. 8 CD spectra and DLS of D313K mutant of cl-Par-4 in presence of various concentration of $MgSO_4$ at pH 7.

Cl^- increases cl-Par-4 solubility more than SO_4^{2-}

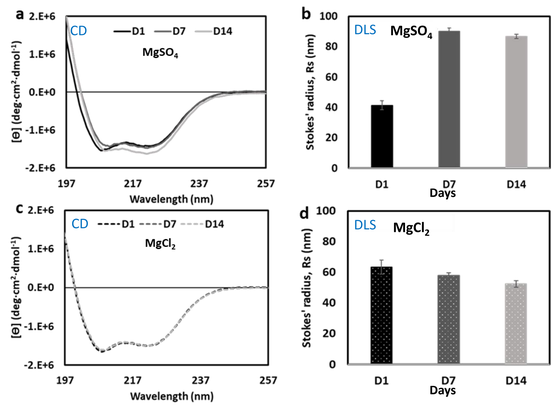

Fig. 9 Time-course CD spectra and DLS of cl-Par-4 in presence of 10 mM $MgSO_4$ (a) & (b); and 10 mM $MgCl_2$ (c) & (d) at pH 4.

CONCLUSIONS

- SAC region as well as linker region of cl-Par-4 possess higher disorder propensity whereas coiled-coil (CC) domain shows ordered propensity
- Both Na^+ & K^+ exert similar effect of cl-Par-4 structure
- Divalent cation, Mg^{2+}, exerts similar effect on cl-Par-4 structure as monovalent cations, Na^+ & K^+, but at approximately five times lower concentration
- Mg^{2+} exerts similar effect on D313K mutant as on wild-type cl-Par-4 structure, but at lower concentration
- cl-Par-4 has better stability in presence of Cl^- than in presence of SO_4^{2-} ions

SIGNIFICANCE

- These findings are helpful to induce a structured conformation of cl-Par-4 that will permit structural determination of this protein via X-ray crystallography or NMR

FUTURE DIRECTION

- Structural determination of cl-Par-4 via X-ray crystallography
- Structural determination of cl-Par-4 via Nuclear Magnetic Resonance (NMR)

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Department of Chemistry and Biochemistry
Old Dominion University, Norfolk, VA 23508, USA