IBEC-SJD INTERNATIONAL PhD PROGRAMME

Position

1. Project Title/ Job Position title:
   Disruption of EWSR1-FLI1 condensates as a therapeutic strategy in Ewing Sarcoma

2. Research project/ Research Group description:

Ewing Sarcoma (EwS) is a rare pediatric disease cytogenetically determined by the chromosomal translocation EWSR1-FLI1. This translocation reorganizes chromatin acting as a pioneer transcription factor (TF) with capacity to open chromatin regions and activate super-enhancers (SEnh). Pharmacological modulation of TF's is still challenging, and direct inhibitors of EWSR1-FLI1 have not yet reached the clinic.

EWSR1-FLI1 fusion protein may undergo phase separation based on low complexity domains (LCD, intrinsically disordered regions). These domains are prone to form condensates that increase the concentration of cofactors, as BRG1 or RING1B, through a very narrow optimal window of LCD interactions. Exogenous expression of EWSR1 LCD increase self-interaction at GGAA repeats while represses endogenous EWSR1-FLI1 transcription (Chong et al., 2022). Thus, condensate disruption with peptides offers a promising new direction for cancer therapy by focusing on the structural disassembly of protein condensates rather than traditional inhibition methods. Here, we aim to employ deep mutagenesis of the LCD of EWSR1 and of the cofactors involved in phase separation to (i) define the residues driving EWSR1-FLI1 condensates formation and, (ii) dismantle the condensates via peptides designed to saturate interactions at these positions.

The research group will be composed by the PI from IBEC, Dr. Benedetta Bolognesi and the PI from IRSJD, Dr. Sánchez-Molina, one PhD student and a master student. The IBEC research group has strong expertise in massively parallel approaches based on Deep Mutational Scanning (DMS) to quantify effects of mutations in a specific protein domain, including disordered regions. They also have experience in designing and optimizing peptide libraries. The IRSJD group has expertise in EwS models and is focused on the development of new treatments for sarcoma patients based on deregulation of chromatin regulation. IRSJD would provide the clinical and biological expertise to develop the appropriate selection methods in EwS in vitro and in vivo models. IBEC would contribute by developing predictive models, building mutational libraries and analyzing the large datasets generated. Together, this collaboration could yield significant advancements in understanding this aggressive cancer and developing novel treatments as well as generating shared tools and databases, benefiting the broader research community in pediatric cancer biology.

3. Job position description:

The PhD student would be responsible for the tasks comprising the two main aims: (i) define the main residues from EWSR1-FLI1 and RING1B implicated in condensation and, (ii) disentangle condensate structures designing peptides that saturate interactions at these positions.

EwS cells remain dependent on the formation of condensates for the reprogramming of cells and consequently cell proliferation. Leveraging from this dependency we would use a deep mutational scanning approach to identify hotspots residues or regions required for condensation and therefore proliferation. In brief, large mutational libraries (1000s of sequence variants) of EWSR1 LCD and of its cofactor RING1B, will be designed and synthetized encompassing multiple mutations in these regions, alone and in combination. After transfections, selective enrichment or depletion of the different sequences will happen by proliferation assays. Deep sequencing of samples taken at different time points will reveal the key residues for proliferation. In this part of the project the student would be in charge of library design proliferation assays, as well as of the validation experiments to confirm alteration of condensation. They will also carry out analyses of the generated sequencing data, thus developing both experimental and computational skills.

Once the main positions involved in condensation are defined, designed peptides encompassing these positions would be overexpressed. These peptides will compete with homotypic intermolecular interactions that normally drive condensate formation enabling condensate disruption. B-isoxazole precipitated condensates in EwS cells are enriched in EWSR1-FLI1, SWI/SNF subunit BRG1 and EWSR1 (Boulay G. et al, 2017). To get deeper insight into the contribution of each region, b-isoxazole precipitations will be performed and analyzed by Western Blotting upon peptide overexpression. Genome wide localization of biomolecular condensates will be further analyzed by DisP-seq methodology in combination with ATAC-seq (Hang-Xing et al. 2024). In this part of the project the student will further develop their skills in biochemistry and sequencing analysis.

Overall, this approach will provide a detailed molecular map of the interactions driving EWSR1-FL1 condensation revealing key insights into the contribution of EWSR1-FLI1 and its cofactors such as RING1B to pathological condensation. It will also result in the rational design of peptides able to disrupt this process and slow down cell proliferation.

Group Leaders at IBEC

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