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OVERVIEW

Our research focuses on a protein complex named cohesin that embraces DNA to mediate sister chromatid cohesion, a process essential for chromosome segregation and faithful DNA repair by homologous recombination. Cohesin also plays a major role in the spatial organisation of the genome by promoting long-range DNA looping, which in turn contributes to transcriptional regulation. Mutations in cohesin have been found in several tumour types, most prominently in bladder cancer, Ewing sarcoma and acute myeloid leukaemia. Germline mutations in cohesin and its regulatory factors are also at the origin of human developmental syndromes collectively known as cohesinopathies.

"We have identified a differential requirement of cohesin-STAG1 and cohesin-STAG2 for NIPBL, a key regulator of cohesin activity and the gene most commonly mutated in cohesinopathy patients."

Our goal is to understand how cohesin works, how it is regulated, and how its dysfunction contributes to cancer and other human diseases. In particular, we are intrigued by the existence of different versions of the cohesin complex. We use human cells and mouse models carrying *knock out* alleles of genes encoding variant cohesin subunits to investigate their functional specificity.

RESEARCH HIGHLIGHTS

NIPBL is not required for loading cohesin on chromatin

The spatial organisation of the genome inside the nucleus is critical for transcription, DNA replication and repair. Cohesin mediates 3D genome organisation by binding to chromatin and extruding DNA loops that become stabilised at several locations along the genome, most notably at sites bound by CTCF. In this way, the complex facilitates contacts between promoters and distal enhancers while restricting such interactions within topological associated domains (TADs). Loop extrusion by cohesin also promotes intermixing of active/inactive chromatin compartments.

There are two versions of the cohesin complex in all somatic vertebrate cells that carry SMC1A, SMC3, RAD21, and either STAG1 or STAG2. Results from our group and others indicate that the two complexes make specific contributions to 3D genome architecture, and further suggest that their different chromatin association dynamics are responsible for these specific functions. In turn, chromatin association is modulated by the interactions of cohesin with its regulators. STAG2 is more often found associated with the unloading factor WAPL, while cohesin acetyltransferase ESCO1 preferentially acts on cohesin-STAG1 at CTCF-bound sites. What it is not known is how the two complexes respond to limited availability of NIPBL.

NIPBL is currently considered the cohesin loader. It activates the cohesin ATPase and is essential for loop extrusion by cohesin *in vitro*. *NIPBL* is an essential gene, and heterozygous mutations have been identified in over 70% of patients with Cornelia de Lange Syndrome (CdLS), the most common developmental syndrome due to cohesin dysfunction. To assess the consequences of NIPBL knock down (KD), we combined a flow cytometry assay that measures chromatin-bound proteins with analyses of genome-wide distribution of cohesin-STAG1 and cohesin-STAG2 by ChIP-seq and of genome contacts by *in situ* Hi-C. Strikingly, we found that cohesin-STAG1 increases on chromatin and further accumulates at CTCF positions after NIPBL knock down, while cohesin-STAG2 diminishes genome-wide. These effects are independent of the presence of the other complex and are epistatic to downregulation of CTCF, ESCO1, or WAPL. Despite the presence of cohesin-STAG1 on chromatin, loop formation is severely impaired. These and additional data support a model in which, contrary to current thinking, NIPBL is not required for association of cohesin with chromatin. However, it is required for loop extrusion, which in turn facilitates stabilisation of cohesin-STAG2 at CTCF positions after being loaded elsewhere (FIGURE 1, right). In contrast, cohesin-STAG1 is loaded and stabilised at CTCF sites even under low NIPBL levels, although in that condition it is unable to form long loops (FIGURE 1, left). These results add to our understanding of the different behaviour of cohesin-STAG1 and cohesin-STAG2. More importantly, they provide a new perspective on the role of NIPBL on cohesin dynamics that needs to be considered when thinking of potential therapies for CdLS.

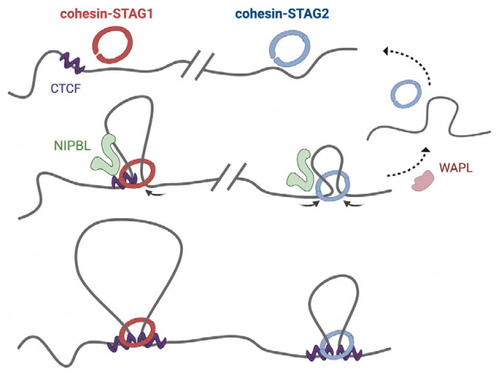

FIGURE 1 Model for the different NIPBL requirement of cohesin-STAG1 and cohesin-STAG2 in the process of DNA loop formation that drives 3D genome organisation. Created with Biorender.com.

Contribution of STAG2 mutations to aggressive Ewing sarcoma

Ewing sarcoma (EWS) is the second most frequent type of bone cancer in children and young adults. It is driven by a fusion protein, most often EWS-FLI1, which alters the gene expression programme of the cell initiating the tumour. It is a highly aggressive cancer with a 5-year survival below 30% in patients that present metastasis. Among the few recurrent mutations identified in EWS, in addition to the oncogenic fusion, are those that inactivate *STAG2*. Importantly, *STAG2* mutations are often present in the most aggressive EWS tumours, suggesting that the loss of cohesin STAG2 may facilitate the acquisition of the aggressive form of EWS.

From the bioinformatic analysis of transcriptomic data from EWS patients and cell lines, we have identified a gene signature dependent on STAG2 loss that correlates with poor survival. We are currently exploring the contribution of these genes to the metastatic phenotype by analysing, both *in vitro* and *in vivo*, the migration and invasion capabilities of EWS cell clones knocked out for these genes (FIGURE 2). In collaboration with E. de Álava (*Hospital Virgen del Rocío-IBIS*, Sevilla), we are also assessing whether they can serve as biomarkers to predict the presence of metastases before their actual detection using immuno-histochemistry (IHC) in tissue microarrays (TMAs) from patient samples. Finally, with the help of Carmen Blanco (Experimental Therapeutics Programme, CNIO), we are carrying out drug screens to identify vulnerabilities in EWS cells lacking STAG2. ■

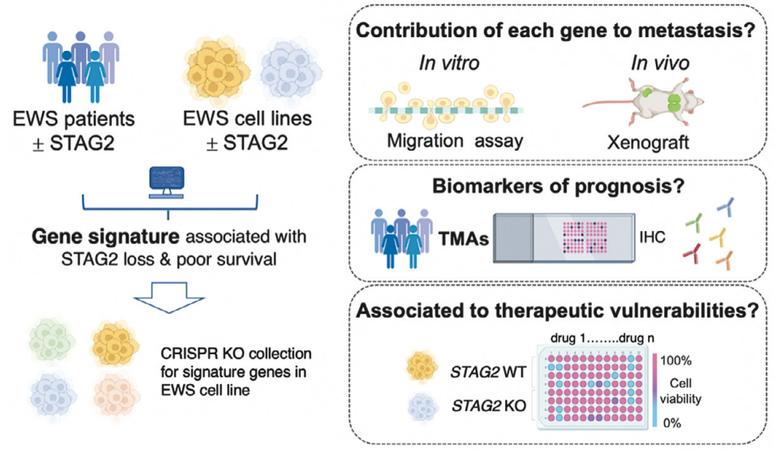

FIGURE 2 Strategy to understand and exploit the contribution of *STAG2* mutations to aggressive Ewing sarcoma.

PUBLICATIONS

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