CHROMOSOME DYNAMICS GROUP

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OVERVIEW

Our research focuses on a protein complex named cohesin, which engages 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, DNA replication and recombination. Two variant cohesin complexes, carrying either the STAG1 or the STAG2 subunit, are present in all somatic vertebrate cells. While cells require a single complex for viability, both are necessary to fulfill embryonic development. Mutations in cohesin genes, particularly in *STAG2*, have been found in several tumour types, including bladder cancer, Ewing sarcoma and acute myeloid leukaemia. Germline mutations in cohesin, and its regulatory factors, are also at the origin of developmental syndromes collectively known as cohesinopathies, such as Cornelia de Lange Syndrome (CdLS). Our goal is to understand how cohesin works, how it is regulated, and how its dysfunction contributes to cancer and other human diseases.

"We have determined how STAG2 loss modifies the chromatin interactome of Ewing sarcoma cells and provided a list of potential biomarkers and therapeutic targets."

RESEARCH HIGHLIGHTS

Contributions of cohesin-Stag1 and cohesin-STAG2 to early embryonic development

Cohesin mediates 3D genome organisation by binding to chromatin and extruding DNA loops that become stabilised at multiple sites 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 topologically associating domains (TADs). Cohesin-STAG1 and cohesin-STAG2 present different chromatin association dynamics that dictate their specific contributions to genome folding.

Changes in genome folding accompany the extensive epigenetic remodelling that takes place upon fertilisation. The poorly defined TADs observed in the zygote only become mature after the 8-cell stage. How the two cohesin complexes contribute to these early stages of embryonic development remains unknown. We have previously reported that *Stag1* or *Stag2* knock out (KO) mouse embryos die by mid-gestation. However, it is unclear whether the defects that cause this lethality are the consequence of alterations at earlier stages. Moreover, these were zygotic KOs, in which maternally deposited *Stag1* and *Stag2* gene products could form functional complexes. We have now used confocal microscopy to analyse the behaviour of STAG1 and STAG2 from the zygote to the blastocyst stage and the relevance of their maternal contribution. We have found clear differences in the relative abundance of the two paralogs at these stages, in part due to the persistence of maternal STAG1 beyond the 2-cell stage (Figure 1). STAG2 accumulation in the more differentiated cells that result from the first cell-fate decision at blastocyst stage was also observed. Importantly, neither paralog is essential for viability when the other is present. However, transcriptome analyses are currently underway to identify alterations that may lead to defects during gastrulation. These studies will help us to understand the molecular pathways driving developmental abnormalities in cohesinopathy patients, particularly those that carry mutations in *STAG1* and *STAG2*.

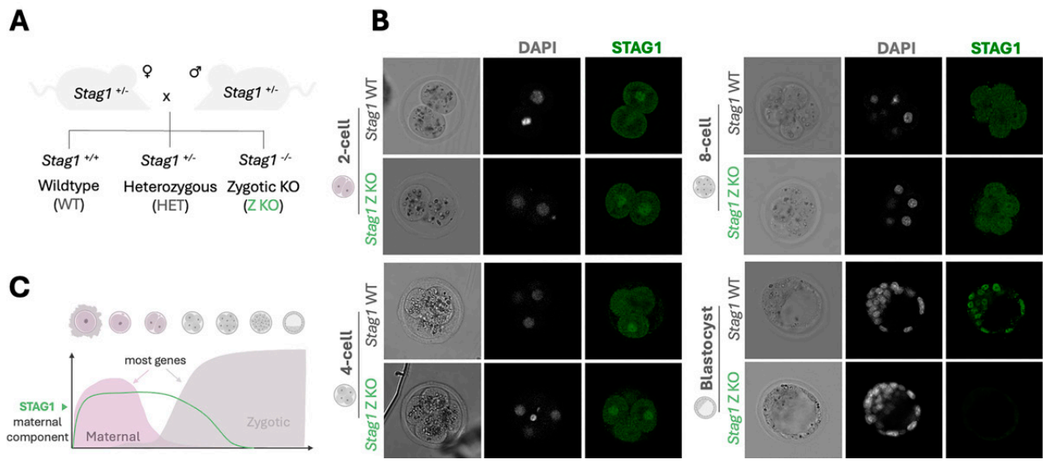

FIGURE 1 Unusual persistence of maternally contributed *Stag1* gene product in early embryos. **A**. Mating scheme to obtain *Stag1* (Zygotic) KO embryos. **B**. STAG1 staining (green) in these embryos and their wildtype (WT) counterparts. **C**. During maternal-to-zygotic transition at 2-cell stage, most maternally deposited gene products are cleared, but not STAG1.

STAG2 loss in Ewing sarcoma rewires chromatin contacts

Ewing sarcoma (EWS) is a paediatric bone cancer driven by a fusion protein, most often EWS::FLI1, which acts as a neomorphic transcription factor. It is a highly aggressive cancer with a 5-year survival rate of less than 30% in patients that present metastasis. The prognosis is generally better for patients with localised tumours at diagnosis, but around 25% of these patients do not respond well to therapy and have poor survival. Among the few recurrent mutations identified in EWS, in addition to the oncogenic fusion, are those that inactivate *STAG2*. These mutations are often present in the most aggressive EWS tumours.

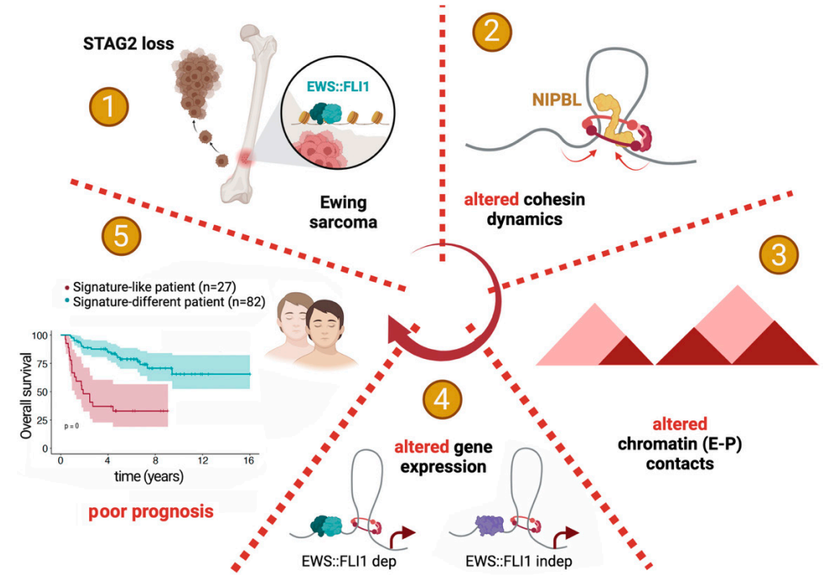

FIGURE 2 Mechanistic understanding of the consequences of STAG2 loss in Ewing sarcoma. Cohesin/NIPBL imbalance upon STAG2 loss in Ewing sarcoma cells alters chromatin contacts, which contributes to altered transcription of oncogene-dependent and -independent genes and promotes an aggressive phenotype.

To understand how loss of cohesin STAG2 facilitates the acquisition of the aggressive phenotype, we generated isogenic EWS cell lines with and without STAG2. Firstly, we note that STAG1 is unable to compensate for STAG2 deficiency and overall levels of chromatin-bound cohesin are severely reduced. In contrast, levels of the cohesin processivity factor NIPBL remain unchanged, likely affecting DNA looping dynamics (Figure 2). Genomic profiling and high-resolution chromatin interaction data, from Capture Hi-C analyses, indicated that cohesin-STAG2 facilitates communication between EWS::FLI1-bound long GGAA repeats, acting as neoenhancers, and their target promoters. Changes in CTCF-dependent chromatin contacts involving signature genes, unrelated to EWS::FLI1 binding, were also identified. Integration of transcriptomic data from patients and cellular models led to the identification of a STAG2-dependent gene signature associated with worse prognosis. These results suggest that STAG2 loss modifies the chromatin interactome of Ewing sarcoma cells resulting in altered transcription of EWS::FLI1-dependent and independent genes. We are investigating how these alterations promote metastasis and/or resistance to chemotherapy. ■

PUBLICATIONS

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