REVEALING GENE FUNCTIONS IN HEALTH AND DISEASE

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Folding in three-dimensional space is a distinct feature of eukaryotic genomes. This highly organized process governs nuclear gene positioning, where transcriptionally-active gene regions locate themselves in interior compartments and inactive heterochromatic regions end up near the periphery. Genes that govern genome organization and nuclear architecture maintenance are crucial for human development, and when mutated they cause a slew of human diseases characterized by developmental defects.1,2

Cohesinopathy is one such syndrome where mutations in the genes encoding the cohesin protein complex disrupt the spatiotemporal dynamics of genome organization. This multi-subunit complex interacts with DNA and has crucial functions in genome folding, cell division, DNA repair, and transcription. Cohesin mutations affect multiple organ systems, and cohesinopathy patients have cardiac and craniofacial abnormalities, such as cleft lip, dental defects, delayed bone ossification, and intellectual disabilities. Despite these known phenotypes, the cellular disease mechanisms remain elusive.3

Neural crest cells are embryonic stem cells that generate different cell types in the embryo, including the craniofacial bones and cartilage.3 To identify the molecular mechanisms linking cohesin function and craniofacial pathogenesis, Ricardo Linares-Saldana and colleagues at the University of Pennsylvania examined mutations affecting neural crest cell development and differentiation.4 Patients with mutations in the bromodomain-containing protein 4 (BRD4) gene presented defects similar to cohesinopathy. BRD4 recruits transcriptional machinery to DNA and acts as a histone acetyltransferase. The researchers investigated interactions between cohesin and BRD4 to understand neural crest cell development in cohesinopathy and related syndromes.

Linares-Saldana and his team generated a mouse model to dissect the relationship between BRD4 and the cohesin machinery in cohesinopathies. Using the Cre–loxP system, they deleted Brd4 selectively in pre-migratory neural crest cells. Immunohistochemistry (IHC) with anti-BRD4 antibody confirmed efficient tissue-specific Brd4 deletion, where most neural crest cells lacked IHC signal. The neural crest cell-specific Brd4 knockdown led to perinatal lethality; the researchers were unable to harvest mutant mouse embryos after birth, which suggested that Brd4 is essential for neural crest cell development and mouse viability. In late-gestation stage mutant embryos, the researchers observed a range of craniofacial defects, including cleft palate, abnormal tooth formation, and loss of middle-ear bones similar to phenotypes common in human patients.

To determine the functional connections between BRD4 and cohesin, the researchers immunoprecipitated BRD4 from murine embryonic stem cells (mESCs) and found several binding partners, including Nipped-B-like protein (NIPBL), a cohesin loading factor. Loss of Nipbl during mouse embryogenesis resulted in similar developmental defects to that observed in the Brd4 mutant embryos. NIPBL is a positive effector of cohesin that regulates genome folding via facilitating cohesin loading onto chromatin. Through chromatin immunoprecipitation (ChIP) sequencing, the researchers found that BRD4 is necessary for NIPBL occupancy on chromatin. Together, these data revealed that BRD4 mediated genome folding and higher-order chromatin organization through NIPBL and cohesin interactions.

To investigate whether loss of the BRD4–NIPBL interaction recapitulated phenotypes observed in cohesinopathies, the researchers differentiated neural crest progenitor cells lacking Brd4 into smooth muscle actin plus (SMA+) cells. While Brd4 knockdown did not affect Nipbl expression, loss of Brd4 hindered progenitor differentiation into smooth muscle. Co-expression of a wildtype human BRD4 construct rescued the progenitor differentiation defect and generated SMA+ cells. In contrast, the introduction of human BRD4 mutants defective in the NIPBL-binding domain did not rescue smooth muscle cell differentiation. This rescue data demonstrated that BRD4 is a positive modulator of NIPBL in maintaining cohesin function during genome folding. Such studies illustrate the molecular and cellular players orchestrating genome folding and the physiological consequences of their dysfunction.

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Immunome checkpoint proteins prevent the immune system from attacking healthy cells in the body. Cancer cells engage this pathway to evade immune surveillance by blocking T cell infiltration into tumors. When T cell receptors recognize and bind to immune checkpoint proteins on tumor cells, the tumor sends an “off” signal to the T cells, which stops the immune system from destroying the cancer. Therefore, blocking T cell receptor binding to checkpoint proteins with inhibitor drugs could entice T cells to kill cancer cells.¹

In recent years, immune checkpoint inhibitors such as anti-program death 1 (anti-PD-1) and anti-cytotoxic T-lymphocyte–associated antigen 4 (anti-CTLA-4) have shown promising results as treatments for a variety of cancers. However, cancer cells employ several resistance mechanisms that make it difficult to fully destroy them in patients. Treatments that combine several immune checkpoint inhibitors help overcome the resistance problem. However, this approach enhances drug toxicity, which leads to therapy discontinuation. Identifying immune mechanisms contributing to drug resistance and toxicity in cancer immune checkpoint inhibitor therapies could help researchers improve patient outcomes.²,³

Immune-related adverse events (irAEs) are autoimmune side effects that damage multiple organs after immune checkpoint drug administration. Immune-related enterocolitis (irEC) in the intestine is the most serious type of irAE, causing bowel perforation, sepsis, and death.² To understand immune pathway mechanisms contributing to irAE, Yared Hailemichael and colleagues at the University of Texas MD Anderson Cancer Center performed a comprehensive analysis of immune system gene expression in irEC tissue obtained from patients.³ This analysis revealed 52 upregulated genes in the irEC tissue compared with normal tissue. Among them, cytokine interleukin-6 (IL-6) showed the highest increase in expression. Moreover, genes known to be induced by IL-6, such as IL-11 and SAA1, were also upregulated.

Based on this upregulated gene expression marker profile, the researchers estimated which immune cell population was predominantly present in the irEC tissue. They identified proinflammatory T helper 17 (Th17) cells and neutrophils in irEC tissues but not cytotoxic CD8⁺ T cells. When the researchers immunostained human intestine tissue samples with T helper cell markers CD3, CD4, and CTLA-4, they found higher infiltration of Th17 immune cells in irEC tissues compared to control tissue.

Next, Hailemichael’s team set out to identify the molecular differences in irEC patients induced by anti-CTLA-4 and anti-PD-1 drug administration. The upregulated gene expression profile remained similar between these two patient cohorts. Both anti-CTLA-4 and anti-PD-1 upregulated IL-6 and IL-11, in addition to genes encoding neutrophil and monocyte chemoattraction. The researchers also found that the amount of Th17 memory cells was significantly higher in irEC induced by anti-CTLA-4 compared to the anti-PD-1 treatment.

Collectively, the patient tissues analyses suggested that the differentiation of proinflammatory Th17 cells triggered cytokine IL-6 release and contributed to irEC in patients receiving anti-CTLA-4 immune checkpoint inhibitor. Similarly, the researchers corroborated this observation in a mouse model where mice with melanoma receiving anti-CTLA-4 therapy also showed significantly increased proinflammatory IL-6 compared with control mice. To test whether IL-6 inhibition could have a beneficial effect by reducing the proinflammatory side effects of the immunotherapy, Hailemichael’s team incorporated IL-6 blockers in anti-CTLA-4-receiving melanoma and colon carcinoma mouse models. The tissues showed increased tumor-infiltrating T cell localization. Remarkably, while no mice from the control groups and only about 20 percent of mice receiving anti-CTLA-4 therapy were alive at 60 days, 58 percent of mice receiving anti-CTLA-4 with IL-6 blockade were alive. Together, these results highlighted the therapeutic benefits of IL-6 blockade in combination with cancer immunotherapies by significantly reducing inflammation–driven side effects.

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Genomes are under constant environmental and intrinsic assaults that generate a variety of detrimental mutations, such as chromosomal rearrangements and double-stranded DNA breaks (DSBs). DNA interstrand crosslinks (ICLs) are highly toxic double-strand lesions that prevent replication and transcription by stopping DNA strand separation. ICLs occur when alkylating agents disrupt covalent bonds between the two complementary DNA strands. Because ICLs affect both strands, their repair requires multiple molecular players to correct the errors and protect cells from the deleterious consequences of mutations.1

The Fanconi anemia (FA) pathway is a crucial player in ICL repair, and disruptions of one or more genes in this pathway cause FA, a rare genetic disease characterized by developmental birth defects, bone marrow failure leading to blood cell deficiency, and cancer predisposition. The multi-step FA ICL repair process recruits the FA core complex composed of several protein subunits that associate with damaged chromatin and ubiquitin machinery to promote ICL repair. Researchers are hunting for downstream effectors that mediate ICL repair along with FA pathway components.2

To identify novel factors involved in ICL repair, Lisa Schubert and her colleagues at the University of Copenhagen carried out a genome-scale CRISPR–Cas9 loss-of-function screen.3 They applied a low dosage of the ICL-inducing drug mitomycin C (MMC) to cultured human cells and surveyed for genes whose knockdown sensitized the cells to MMC and affected their survival. In addition to FA pathway proteins, the screen revealed a new molecular player—Suppressor of Cancer Cell Invasion (SCAI)—whose down-regulation made the cells sensitive to MMC. The researchers also found that SCAI-deficient cells were sensitive to Cisplatin, another ICL inducer, which suggested that the cells were unable to repair ICLs induced by these drugs. When the researchers expressed SCAI in the cells lacking SCAI, they fully rescued the drug sensitivity phenotype.

SCAI is a transcription corepressor associated with several diseases such as spinocerebellar ataxia and hypoglycemia. To explore SCAI function during ICL repair, Schubert’s team performed another complementary genome-scale CRISPR–Cas9 screen using SCAI-deficient cells and identified regulators that suppressed the cells’ hypersensitivity to MMC. Among the positive hits, the inactivation of several FA pathway proteins in cells lacking SCAI increased their resistance to MMC. The researchers further validated these results by silencing the FA pathways genes FANCA and FANCD2, which restored MMC exposed SCAI-deficient cells’ survival and suppressed ICL accumulation. These results indicated that SCAI orchestrates ICL repair through the FA pathway.

The researchers also identified other molecular factors participating in the ICL repair pathway. Using SCAI immunoprecipitates and mass spectrometry, they analyzed protein components that physically interacted with SCAI. Consistent with the screening data, SCAI immunoprecipitates were enriched with many FA pathway proteins. In addition, the researchers also found enrichment of all five subunits of the polymerase ζ (Polζ) complex in the immunoprecipitates.

Schubert’s group then assessed the mechanism of SCAI-mediated DSB repair by analyzing DSB repair products in SCAI-deficient cells using next-generation sequencing. The sequencing results revealed the predominant accumulation of a peculiar deletion around the ICLs that was present only in the absence of SCAI. Based on these DSB intermediates, the authors hypothesized that SCAI regulates the choice between homologous recombination and microhomology-mediated end-joining repair pathways during ICL repair. Together, these findings indicated the critical function of SCAI in ICL repair through the FA pathway.
Organ transplantation is the only available treatment for patients with severe, chronic diseases that lead to organ failure. However, there is a shortage of organ donors, which creates long wait times while finding a donor. With increasing organ donor shortages and patient deaths, there is an urgent need for alternate clinical approaches. Cell transplantation is an exciting avenue in medicine that could offer respite to patients with organ failure, cure diseases, and provide regenerative abilities to injured tissues. For example, transplanted hepatocytes with regenerative abilities show potential to treat liver diseases. Additionally, in type 1 diabetes, transplanted cells could function as a drug reservoir for replenishing insulin.¹

While cell transplantation offers tremendous opportunities for clinical use, the technology is still in its infancy. Researchers combine multidisciplinary tissue engineering approaches to develop biomaterial scaffolds with cells of interest. Cells embedded in a variety of biomaterials form tissues and integrate within the host environment such that they do not trigger immune activation and survive for a long time in the host.²

Biomaterials with unique mechanical and biochemical properties are quickly integrating into cell transplantation applications, improving clinical outcomes. They protect cells, improve cell survival and migration, and minimize interaction with immune cells. Developing a pouch from biomaterials that encases cells and allows nutrients, gases, and relevant biological molecules to permeate while shielding cells from immune detection is fundamental to biomaterial-based device design.²

Sajeesh Perikamana and colleagues at the Duke University School of Medicine developed a cell transplantation device made of the polysaccharide chitosan.³ The device contained an adhesive inner and a repulsive outer surface carrying a reservoir to house cells. The researchers rigorously tested molecule permeability across the chitosan wall and found that the chitosan pouch was permeable to nutrients, oxygen, and cell-secreted molecules, such as insulin and albumin.

Biomaterial degradation in the human body is a limiting factor in cell transplantation studies. To improve the stability of the chitosan pouch, the researchers modified its outer surface with 1,12-dodecanedioic acid (DDA) molecules that created an outer layer resembling a bristle brush. When they incubated these pouches with a polysaccharide-degrading lysozyme solution, the DDA-modified chitosan membranes showed minimal degradation compared to the corresponding unmodified chitosan membranes. The researchers further validated that the DDA modification did not change the diffusion properties of the chitosan pouches for nutrients and low molecular weight compounds.

Biomaterials often attract cellular and protein deposits, limiting their use in cell transplantation applications. To examine whether DDA modification could reduce cell and protein accumulation in vivo, the researchers implanted unmodified and DDA-conjugated chitosan pouches into the subcutaneous space of immunocompetent mice. When the scientists retrieved these chitosan membranes 3 days post-implantation, they found a thick layer of DAPI+ cells on the unmodified chitosan membranes. In contrast, the DDA-modified chitosan membranes showed significantly less cell deposition on the surface.

Lastly, the researchers tested the clinical utility of these pouches in vivo. They implanted unmodified and DDA-modified pouches carrying human primary hepatocytes in the subcutaneous layer of immunocompetent mice. Using ELISA-based kits for human hepatocyte secretory protein albumin, they monitored the viability and function of the transplanted hepatocytes and found that the device supported cells for up to 6 months. Quantification of the human albumin in the peripheral blood revealed that while both the cohorts produced albumin from the transplanted cells, the animals that received DDA-conjugated pouches had significantly higher levels compared to those that received unmodified chitosan pouches.

Together, the development of biomaterial-based cell transplantation devices supported human hepatocyte viability without triggering an immune response, and chemical modifications on these devices can further improve their efficacy.

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References

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**Article 2: Mitigating Cancer Immunotherapy Side Effects**

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**Article 4: Developing Biomaterial for Cross-Species Cell Transplantation**
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