Int. J. Dev. Biol. 69: 161-172 (2025)
https://doi.org/10.1387/ijdb.250129km

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Sialylated glycoproteins and sialyltransferases involved in mesoderm-derived organ formation during embryogenesis

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ABSTRACT Most major organs, like muscles, bones, vessels and kidneys, develop from the mesoderm, one of three germ cell layers in triploblastic organisms. Sialic acids significantly affect embryonic development by regulating cell division, migration and death through signaling pathways and cell adhesion, which support morphogenesis. Loss of early biosynthetic enzymes reduces embryonic viability and leads to complex phenotypes, while the loss of terminal enzymes primarily results in tissue-specific defects in mesoderm-derived organs. Key sialylated glycoproteins involved in the developmental processes of mesoderm and mesoderm-derived organs have been identified across various species as major effectors. These enzymes and glycoproteins are of significant interest and are discussed in the present review.

KEYWORDS: sialic acids, sialyltransferases, mesoderm, embryonic development, organogenesis

Introduction

A dense complex glycocalyx, composed of numerous glycoconjugates including glycoproteins and glycolipids, coats cell surfaces. Sialic acids represent the predominant terminal sugar residues of glycoconjugates found on most cells' surfaces. Sialic acids consist of a group of nine-carbon amino sugars that are positioned at the nonreducing terminal ends of *N*- or *O*-glycoproteins and glycolipids, forming the so-called sialome (Cohen and Varki, 2010; Sato and Kitajima, 2021; Varki, 2017). Their high structural diversity has been shown to participate in various cellular functions during different stages of embryonic development.

Following fertilization and cleavage, the mesoderm, ectoderm, and endoderm form and rearrange during gastrulation, involving cell movement, interactions, and regulation of adhesion factors via ligand-receptor signals involving Sia. Additionally, epithelial-mesenchymal transition (EMT) occurs, alongside the regulation of sialylation. Most major organs develop afterwards during early and late organogenesis from the mesoderm, where sialylation influences the formation of structures, including the skeleton (cartilage and bones), muscles (skeletal and cardiac), kidneys (pronephros and metanephros), the blood and lymphatic vasculature (endothelial and lymphatic vessels). Although the function of sialylation in ectoderm and nervous system development has been thoroughly investigated (see Schnaar *et al.,* 2014; Sato and Kitajima, 2021), its role in the formation of endoderm and particularly in mesoderm and mesoderm-derived organs has received less attention in current reviews.

This review discusses the roles of biosynthetic enzymes and sialyltransferases in the formation of the mesoderm and its derived organs, as well as potential effectors regulated by Sia. Disruption of early cytosolic enzymes in the sialic acid biosynthesis pathway, such as the glucosamine UDP-GlcNAc-2-epimerase/*N*-acetylman-

Abbreviations used in this paper: AcCoA, acetyl-coenzyme A; CMAH, N-acetylneuraminic acid hydroxylase; CMAS or CSS, CMP-sialic acid synthetase; CMP, cytidine monophosphate; dpf, days post-fertilization; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; GNE/MNK, glucosamine UDP-GlcNAc-2-epimerase/N-acetylmannosamine kinase; hpf, hours post-fertilization; Kdn, 2-keto-3-deoxy-D-glycero-D-galacto-nonulosonic acid; ManNAc, N-acetylmannosamine; NANP, N-acetylneuraminate-9-phosphatase; NAL, N-acetylneuraminate lyase; NANS, Neu5Ac-9-P synthase; Neu, neuraminic acid; NEU, neuraminidase; Neu5Ac, N-acetylneuraminic acid; Neu5Gc, N-glycolylneuraminic acid; NCAM, neural cell adhesion molecule; PSA, polysialic acids; Sia, sialic acids; sLex, sialyl-Lewis(x); sLea, sialyl-Lewis(a); ST8Sia2, Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 2; ST8Sia4, Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 4; UDP, uridine diphosphate.

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Submitted: 29 August, 2025; Accepted: 19 February, 2026; Published online: 31 March, 2026.

ISSN: Online 1696-3547, Print 0214-6282

nosamine kinase (encoded by the *GNE* gene), can result in death or improper development of both ectodermal derivatives and mesoderm-derived organs (Varki and Schauer, 2009). Knockouts, knockdowns, and *in vitro* studies have identified key enzymes for sialic acid biosynthesis in mesoderm-derived organs. However, many sialylated effectors and sialyltransferases remain unclear due to their complex and potentially compensatory roles. These components may be valuable for understanding and treating diseases, but further research is needed to define their functions and regulatory networks.

Sialylated glycoconjugates are characterized by a high structural diversity

Sialic acids are derived from *N*-Acetylneuraminic acid (Neu5Ac) and 2-keto-3-deoxynononic acid (Kdn). Neu5Ac and 5-*N*-glycolylneuraminic acid (Neu5Gc). The two most abundant are Neu5Ac and 5-*N*-glycolylneuraminic acid (Neu5Gc) are the most abundant forms, followed by Kdn and neuraminic acid (Neu), which is almost not present in nature (Schauer, 1982). The diversity of sialoglycoconjugates arises from the combination of sialic acid structures, substitutions, degree of polymerization (DP), linkages, and the nature of the related glycans. Hydroxyl or amino groups can be substituted with acetyl, glycolyl, methyl, lactyl, phosphate, or sulfate groups, generating more than 50 structurally different derivatives (Schauer and Kamerling, 2018). Notably, acetyl and sulfate groups play a crucial role in vertebrate embryos (Ertunc *et al.*, 2022; Varki and Schauer, 2009; Varki *et al.*, 1991), Fig. 1A.

Another source of diversity is the different glycosidic α-linkages that connect sialic acid moieties to other glycan units. These linkages vary between α2,3 and α2,6 linkages to galactose-containing glycans, as well as in α2,6 linkages to *N*-acetylgalactosamine (GalNAc) and *N*-acetylglucosamine (GlcNAc) residues. Distinct α2,8 linkages create sialic acid chains on certain glycoproteins, which can vary in their degree of polymerization (DP). Polysialic acid (PSA), in particular, is defined by having a DP greater than 8 (Sato and Kitajima, 2013; Troy, 1992), Fig. 1B. Finally, sialoglycoconjugates can assemble into cell surface micro-domains relaying biological signals (Sato and Kitajima, 2013; Troy, 1992).

Sialic acids are present in Metazoa such as Echinoderms (Miyata *et al.*, 2006), Hemichordata, Cephalochorda, Vertebrata, Platyhelminthes, Cephalopoda and Crustacea. Additionally, they are reported in viruses, bacteria, protozoa, and fungi (Ghosh, 2020). While sialylation occurs in *Drosophila* embryos (Aoki *et al.*, 2007; Frappaolo *et al.*, 2017), evidence of sialylation in other insects is controversial, despite the detection of sialyltransferase homologs in their genome sequences (Kajiura *et al.*, 2015; Koles *et al.*, 2004; Paschinger and Wilson, 2019). C. *elegans* glycans lack sialic acids (Bacic *et al.*, 1990). In addition, Neu5Gc is absent in humans and monotremes (including the platypus) and occurs only rarely in Sauropsids (birds and reptiles), (Altman and Gagneux, 2019; Chen and Varki, 2010). Deaminated neuraminic acid, or Kdn, is found abundantly in lower vertebrates and bacteria (Ghosh, 2020).

Sialic acid biosynthesis, a multistep pathway in embryos

The *de novo* biosynthesis of sialic acids originates from glucose metabolism and occurs through a series of steps located in different cellular compartments. Enzymes involved in the modification of sialic acids and sialyltransferases, which are important for the formation of mesoderm and mesoderm-derived organs, are highlighted in Fig. 1C.

The process begins in the cytosol, by epimerization and phosphorylation of UDP-GlcNAc (uridine diphosphate *N*-acetylglucosamine) into ManNAc-6-P (*N*-acetyl-mannosamine 6-phosphate) and UDP (uridine diphosphate) by the bifunctional enzyme UDP-GlcNAc-2-epimerase/*N*-acetylmannosamine kinase (encoded by the *GNE/MNK* gene). ManNAc-6-P is then condensed with phosphoenolpyruvate (PEP) by the enzyme Neu5Ac-9-P synthase (NANS) to produce *N*-acetylneuraminate 9-phosphate (Neu5Ac-9-P). This compound is subsequently dephosphorylated by *N*-acetylneuraminate-9-phosphatase (NANP) to generate Neu5Ac.

In the nucleus, CMP-Sia (cytidine monophosphate sialic acid) is catalyzed by the cytidine monophosphate *N*-acetylneuraminic acid synthetase (CMAS). The activated CMP-Sia is then transported within the Golgi apparatus, where sialylation occurs (Lepers *et al.*, 1989; Lepers *et al.*, 1990) using a CMP-Sia Transporter (CMP-SiaT)/SLC35A1, a nucleotide sugar transporter. Glycosidic linkages are formed by specific α2,3-, α2,6- or α2,8-sialyltransferases from four families of sialyltransferases ST3Gal, ST6Gal, ST6GalNAc and ST8Sia (Harduin-Lepers *et al.*, 2005; Chang *et al.*, 2009; Teppa *et al.*, 2016; Harduin-Lepers, 2023). Sialyltransferases detected in mesoderm and mesoderm-derived organs are shown in Fig. 1D. These enzymes belong to the glycosyltransferase family 29 (GT29), as documented in the Carbohydrate Active Enzymes database (CAZy, http://www.cazy.org) (Lombard *et al.*, 2014).

O-acetylation and *O*-sulfation of sialic acids occur in the Golgi. The 9-*O*-Acetylation of sialic acids (Sia9Ac) arises at the CMP-Sia level using acetyl-coenzyme A (AcCoA) as a donor. It occurs in conjunction with Golgi-resident sialyltransferases. 8-*O*-sulfation of sialic acids employs *O*-sulfotransferases Wscd1 and Wscd2 after CMP-Sia transport into the Golgi lumen, sialyltransferases, and 3'-phosphoadenosine 5'-phosphosulfate (PAPS).

Other glycosyltransferases from the α-1,6-mannosylglycoprotein 6-β-*N*-acetylglucosaminyltransferase (MGAT) family and hexosyltransferases found in the Golgi apparatus, that generate branched glycans composed of *N*-acetyllactosamine (LacNAc) are ending with Sia, and involved in mesoderm and mesoderm-derivatives formation. The MGAT1 gene encodes *N*-acetylglucosaminyltransferase I (GlcNAc-TI), which initiates complex and hybrid *N*-glycan synthesis (Schachter, 2000). GlcNAc-TI allows mature N-glycoproteins to carry oligomannosyl with branched antennae that contain Sia.

Sialyl Lewis^x (sLe^x) also known as cluster of differentiation 15 (CD15) or stage-specific embryonic antigen 1 (SSEA-1), is a sialic acid tetrasaccharide carbohydrate attached to *O*-glycans, and composed by fucose and *N*-acetyllactosamine (5-acetylneuraminyl-(2-3)-galactosyl-(1-4)-(fucopyranosyl-(1-3))-*N*-acetylglucosamine, Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAcβ). Another tetrasaccharide Sialyl-Lewis^a (sLe^a), sialylated by an α2,3 linkage, differs in its linkages: it is a fucose group linked to *N*-acetylglucosamine by an α1,4 linkage instead of α1,3 linkage for sLe^x, while the galactose is linked to *N*-acetylglucosamine by a β1,3 linkage for sLe^a while in sLe^x it is linked by an β1,4 linkage. The biosynthesis of sLe^x in humans involves fucosyltransferases (CAzy GT10), notably FUT6 and FUT7 the main enzymes, FUT5 and FUT4 to a lesser extent, and FUT9 with a broad substrate specificity. FUT3 synthesizes sLe^a through an α1,4 linkage addition of fucose moieties, whereas

FUT5 only performs it to a lesser extent. Fucosyltransferase activity occurs after the α2,3 linkage of sialic acids by various ST3Gal enzymes (Carvalho *et al.*, 2010; Mondal *et al.*, 2018). Tissues of mesodermal origin also express the Sialyl Tn antigen, an α2,6-sialylated *N*-acetylgalactosamine (GalNAc) linked by a glycosidic bond to serine or threonine. Terminal linkages and sialylated motifs found in mesoderm-derived organs are depicted in Fig. 1D.

Free sialic acids can be recycled following cleavage by neuraminidases/sialidases (NEU) which have distinct cell expression patterns. NEU1 is located in the lysosomes of Metazoa, where it hydrolyses exogenous sialoglycoconjugates. Four human NEU enzymes, classified in the glycoside hydrolase family GH33 according to the CAZy classification, have been described with specific expression patterns (Monti *et al.*, 2010). Neu5Ac is then transported to the cytosol via a sialin/SLC17A5 transporter for the synthesis of CMPNeu5Ac. Cytosolic sialic acid catabolism occurs with *N*-acetylneuraminate lyase (NAL) acting on Neu5Ac to produce ManNAc (*N*-acetyl-mannosamine) and pyruvate (Schauer *et al*., 1999). ManNAc, can be converted into N-acetylglucosamine (GlcNAc) by N-acetylglucosamine 2-epimerase (encoded by the *GNE* gene), and subsequently phosphorylated to GlcNAc-6-phosphate, allowing it to reenter the hexosamine biosynthesis pathway (Varki *et al.*, 2015). The availability and synthesis of sialic acids influence the activity of sialyltransferases (Bork *et al.*, 2017). Despite this, the regulation of Sia, its metabolites, lysosomal recycling, and catabolism remain unclear.

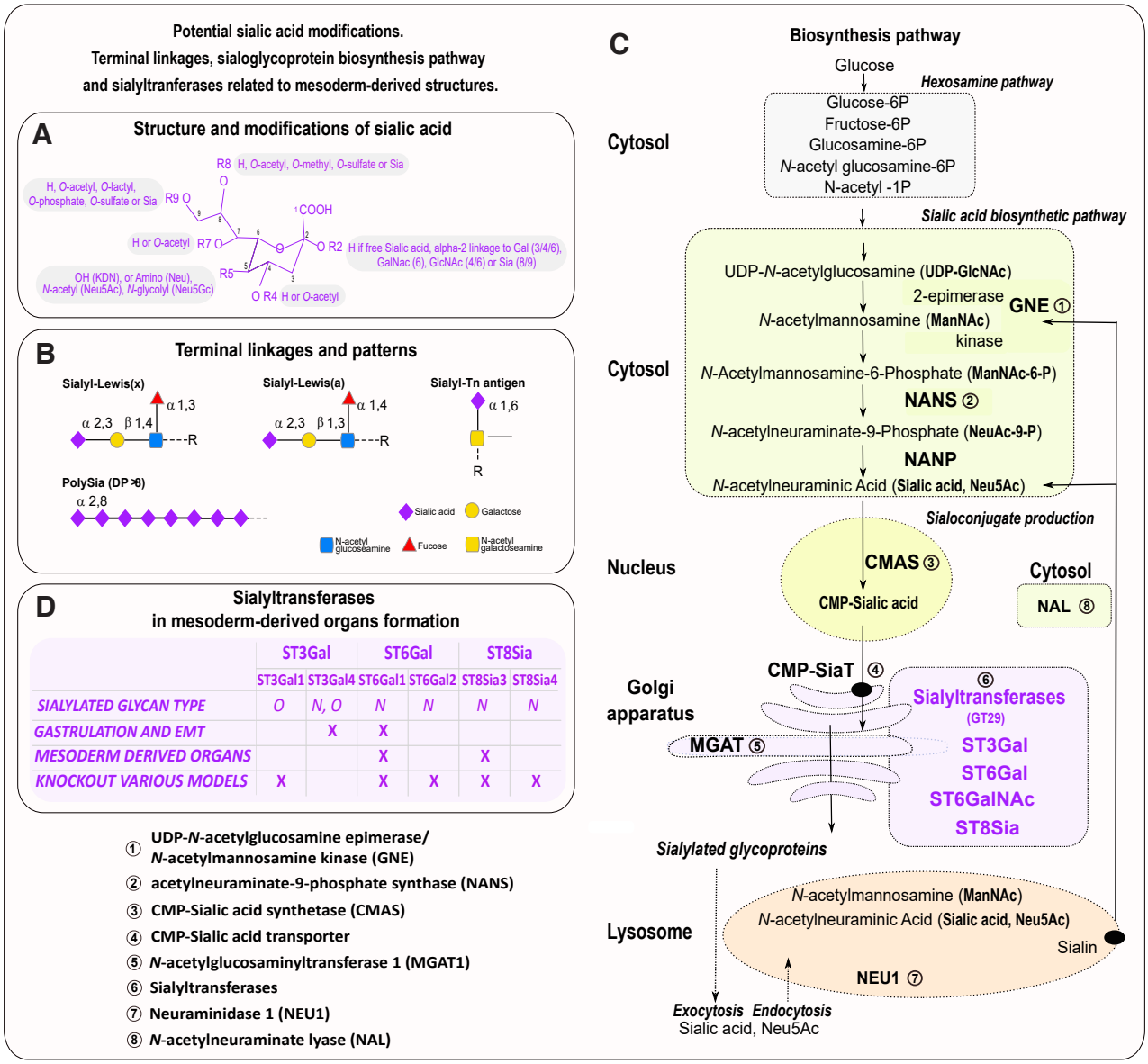

Fig. 1. Sialoglycoprotein biosynthesis pathway, sialyltransferases involved in mesoderm-derived organs, and sialic acid structure. (A) Sialic acid structure and modifications. **(B)** Terminal linkage and patterns found during embryonic development in mesoderm-derived organs. **(C)** Sialic acid biosynthesis pathway and associated enzymes detected in mesoderm-derivatives; the enzymes involved in mesodermal organs formation are in bold. **(D)** Sialyltransferases involved in gastrulation, epithelial to mesenchymal transition (EMT), and mesoderm-derived organs formation in various species. The color refers to the intracellular location of enzymes as follows: cytosol (green), nucleus (yellow), Golgi (orange), and lysosome (pink). Sialyltransferases are purple.

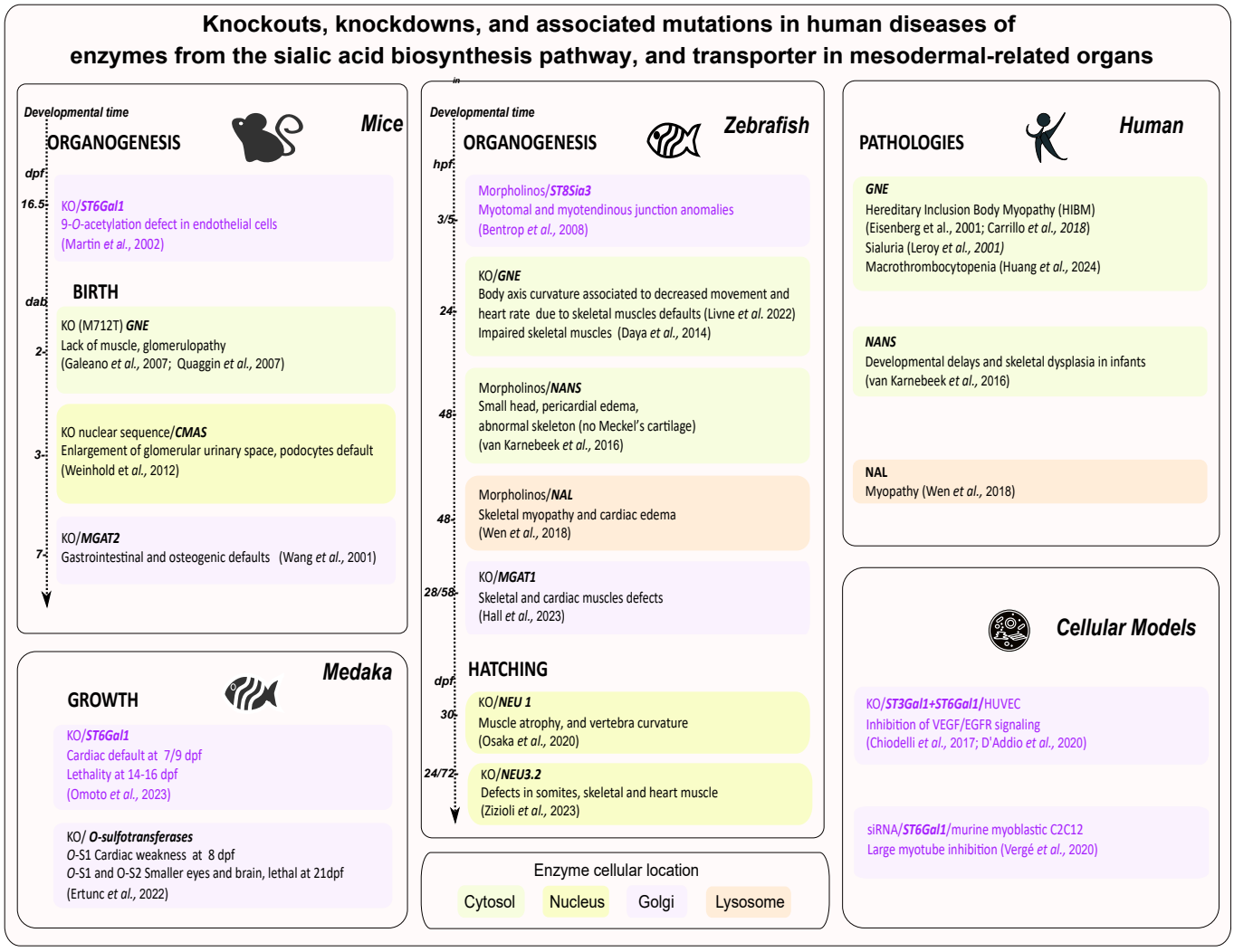

Fig. 2. Enzymes from the sialic acid biosynthesis pathway or transporter shown to be involved in the formation of mesoderm-derived organs and associated mutations in human diseases. For mice and zebrafish, the time of the phenotype's appearance within the developmental program is chronological. For humans, mutations generating congenital diseases are mentioned. The background colors refer to the cellular location of mutated gene products: cytosol (green), nucleus (yellow), Golgi (orange), and lysosome (pink). Sialyltransferases are purple. Abbreviations: Days after birth (dab), days post fertilization (dpf), hours post fertilization (hpf), knockout (KO), knockdown (morpholinos).

Enzymes from the sialic acid biosynthesis pathway linked to mesoderm-derived organs in different species, as evidenced by knockout, knockdown, or related human diseases, are discussed below and summarized in Fig. 2.

Properties of sialic acid in cellular functions

Throughout development, various molecular effectors, including growth factors, receptors, channels, and adhesion molecules, orchestrate a spatiotemporal coordinated decisional program to regulate cellular division, migration, and subsequent morphological changes. Sialic acids have the potential to modulate intracellular signaling and cellular interactions, acting as "fine-tuners" of these processes, due to their negative charge and hydrophilicity, which make them anti-adhesive glycotopes. For instance, sialic acids on endothelial cell surfaces generate repelling electrostatic fields, enabling cell separation and central lumen formation in blood vessels (Abeln *et al.*, 2019). The negative charge of sialic acids on cadherin enhances membrane repulsion of adjacent cells, thereby regulating cell adhesion (Born and Palinski, 1985; D'Addio *et al.*, 2020; Varki and Gagneux, 2012).

Sialylated glycoproteins deliver external stimuli to cells, relay signals between cells, and facilitate communication during mesoderm formation at gastrulation and mesoderm-derived organ development. Sialic acids regulate receptor and channel activity by altering electrostatic interactions, either inhibiting or activating specific molecules: either negatively through negative repulsive charges of sialic acids between two opposite molecules such as for the Neural adhesion molecules (NCAM) (Galuska *et al.*, 2010), or positively by an attractive force between the negatively charged sialylated vascular endothelial growth factor-A (VEGF) and positively charged amino acids to reinforce the activation of VEGFR2 receptor (Chiodelli *et al.*, 2017). Sialic acids silence the hyaluronan receptor LYVE-1 in the lymphatic endothelium (Nightingale *et al.*,

2009), impact receptor oligomerization as evidenced by the effect of α2,6-Sia on endothelial cell adhesion molecule (PECAM1/CD31) to modulate cell adhesion, and play a role in angiogenesis (Kitazume *et al.*, 2010). Sialic acids and PSA also directly bind small cationic molecules, such as growth factors like fibroblast growth factor (FGF2) (Ono *et al.*, 2012; Sato and Kitajima, 2013; Zhang *et al.*, 2004), thus influencing their signaling at gastrulation or during endothelial vessels formation. Furthermore, polysialic acids (PSA) enhance FGFR signaling and cell migration (Li *et al.*, 2011). Interestingly, long chains of sialic acids also control channel activity. PSA regulate the α subunit of the voltage dependent sodium channel influencing the rapidity of closure and opening In rat embryonic myocardium cells (4 to 5 days post fertilization), (Stocker and Bennett, 2006). Finally, sialylation governs glycoprotein stability and influences cell fate. For example, sialylated β1 integrin suppresses cell adhesion and protects cells against apoptosis (Varki and Schauer, 2009). This protection is essential for cells to undergo an epithelial-to-mesenchymal transition during gastrulation, which is necessary for mesoderm formation and subsequent organogenesis.

Mesoderm formation at gastrulation and the epithelial-to-mesenchymal transition

The intermediate cell layer in triploblastic metazoan embryos, mesoderm, is specified during gastrulation, when mesodermal precursors are internalized. This process varies among species. In invertebrates and anamniotes, mesoderm forms through blastoderm folding, as observed in echinoderms, or by rolling, as in amphibians. In amniotes, including birds and mammals, mesodermal cells undergo ingression through the primitive streak (see Nakaya Y., 2008). An epithelial-to-mesenchymal transition (EMT) occurs where cells lose their epithelial traits, modify adhesion by disrupting intercellular contacts and downregulating E-cadherin, and migrate as primary mesenchyme (Hay, 2005). Later, they become secondary epithelial cells and can undergo another EMT to form connective and body tissues. Cycles between EMT and its reverse mesenchymal-to-epithelial transition (MET) also occur during gastrulation and ontogeny (Li *et al*., 2021; Newton AH, Smith CA, 2024; Thiery *et al*., 2024). Sialylation significantly influences EMT as proven in tumors (Du *et al*., 2015). Fig. 3 illustrates the enzymes from the sialic acid biosynthetic pathway involved in gastrulation across various species.

In deyolked zebrafish embryos, the profiles of *N*-glycan showed stage-dependent variations of complex- and hybrid-type glycans. The presence of sialic acids is detected as early as 7 hpf and increasing at later developmental stages. Complex- and hybrid-type glycans are minor components, and their antenna structures are mainly sialyl LacdiNAc (Siaα2-6GalNAcβ1-4GlcNAc), (Hanzawa *et al.*, 2017). Mucin-type *O*-glycans are also detected by 7 hpf (Flanagan-Steet and Steet, 2013). *ST6GAL2* and *ST6GAL2*-related genes are expressed at the onset of gastrulation in the mesendoderm (Petit *et al.*, 2010).

In *Xenopus*, approximately 40% of glycans are sialylated by the end of gastrulation (stage 13), (Qu *et al.*, 2020). The expression of MGAT1 is essential for the synthesis of complex- and hybrid-type *N*-glycans present in high quantities during early gastrulation (stage 10.5) (Onuma *et al.*, 2013). Gastrulating embryos synthesize Sia-mucin in their extracellular matrix (Johnson, 1977). Regenerating half-embryos showed upregulation of ST3Gal4 at gastrulation, indicating a potential role for this enzyme in pluripotency loss (Sosa *et al.*, 2019).

During differentiation into early progenitor cells of the three embryonic germ layers, human pluripotent stem cells become polysialylated. In differentiated mesoderm and endoderm, NCAM is the primary protein that undergoes polysialylation. Of the two polysialyltransferases responsible for adding polysialic acid chains to target proteins, ST8Sia4 is transcriptionally activated by the Goosecoid network during early mesendoderm differentiation, highlighting this polysialyltransferase gene as a contributor to germ layer formation (Berger *et al.*, 2016).

Organogenesis of mesoderm-derived organs

Mesoderm-derived cells give rise to a large numbers of internal organs in vertebrates, excluding the nervous system (ectoderm) and digestive/respiratory systems (endoderm). Cells of mesodermal origin are abundant throughout vertebrates and represent one of the widest varieties of cell types. They arise from lineages including the paraxial mesoderm (axial skeleton, cartilage, muscle, dermis), intermediate mesoderm (urogenital system), and lateral plate mesoderm (body wall, limbs, cardiovascular system).

In amniotes, extraembryonic mesoderm produces membranes like the yolk sac, amnion, and chorion. (Ferretti and Hadjantonakis, 2019). Noticeably, mesodermal derivatives are also associated with non-mesoderm-derived structures, such as smooth and vascular smooth muscles found in the wall of the gastrointestinal tract (Le Guen *et al.*, 2015), endothelial and mesothelial cells, pericytes, alveolar cells, and lipofibroblasts lining the endothelia of the respiratory tract (Herriges and Morrisey, 2014) that are not discussed in the review.

Early morphogenesis frequently begins prior to the completion of gastrulation, and organ development may extend beyond this stage, making it challenging to confine organ morphogenesis strictly to the embryonic period. In aquatic species, organogenesis of mesoderm derivatives typically encompasses both early and later phases, continuing until hatching occurs. In mammals, mesoderm-derived organogen-

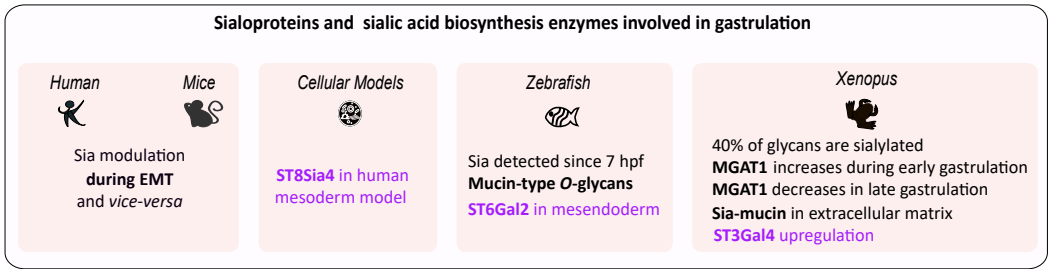

Fig. 3. Sialoglycoproteins and enzymes from the sialic acid biosynthesis pathway involved in gastrulation and epithelial to mesenchymal transition (EMT). Sialyltransferases are highlighted in purple.

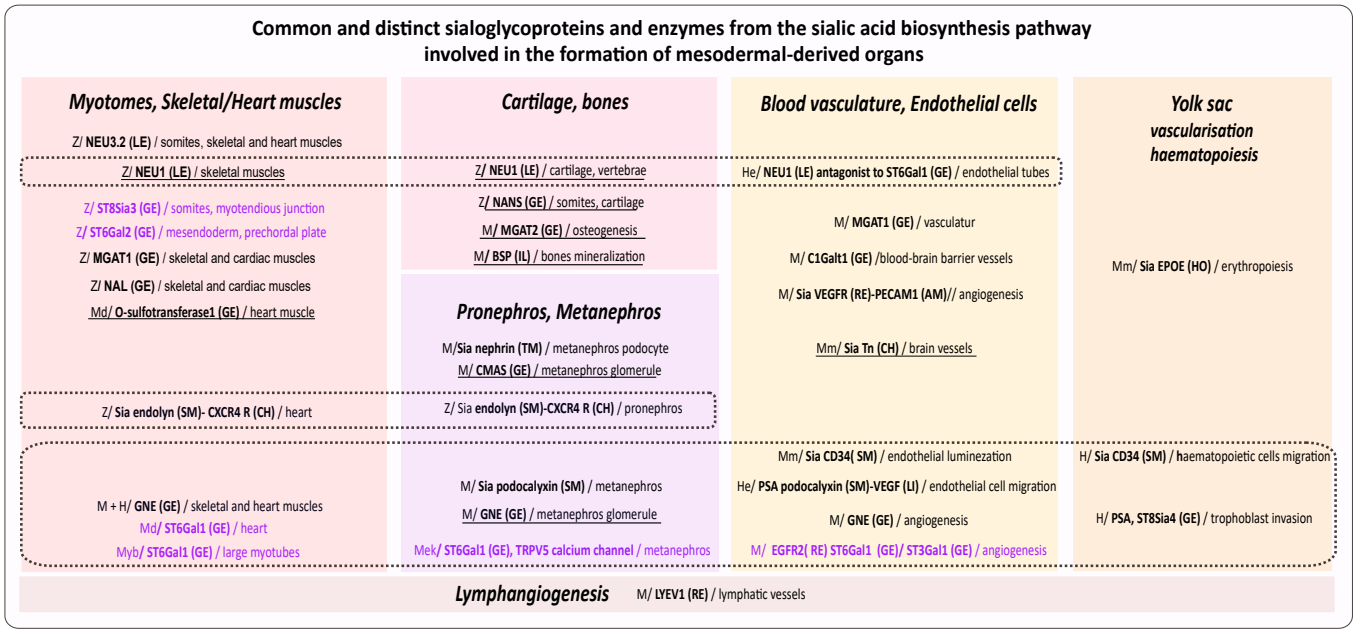

Fig. 4. Common and distinct sialoglycoproteins and enzymes from the sialic acid biosynthesis pathway involved in the formation of mesoderm-derived organs. Species and models: H: human; He: human endothelial cells; M: mouse; Md: medaka; Mek: mouse embryonic kidney cells; Mm: mammals; Me: mouse embryoids; Myb: mouse myoblasts; Z: zebrafish. Effectors: **AM:** adhesion molecule; **HO:** hormone; **CH:** chemokine; CY: cytokine; **GE**: Golgi enzyme; **IL:** integrin domain; **LE**: lysosome enzyme; **RE**: receptor; **SM**: sialomucin; **TM:** transmembrane protein. Sialyltransferases are purple. Dotted lines demarcate common effectors. Underlined effectors act during late organogenesis (In zebrafish organogenesis starts at 10 hpf and proceeds until hatching at 72 hpf (Hisaoka and Battle, 1958; Kimmel *et al.*, 1995), in medaka it begins at 1 dpf and lasts until hatching at 9 dpf (Iwamatsu, 2004). In mammals, embryonic organogenesis continues with fetal growth until birth. In mice, it begins at 7-8 dpf and continues until 12.5 dpf, followed by fetal growth until 20 dpf (Dyban *et al.*, 1991). In humans, embryonic organogenesis starts at 21 dpf and progresses until approximately 53-58 dpf (8 to 9 weeks post-fertilization) (O'Rahilly and Müller, 2010).

esis is succeeded by sustained fetal growth up to birth. Fig. 4 summarizes sialylated glycoproteins and enzymes from the biosynthesis pathway that are critical for the development of various mesoderm-derived organs across species during the embryonic and early postembryonic periods.

Cartilage and bones

Among the enzymes involved in sialic acid synthesis, NANS is not only essential for brain development but also for skeletal formation. NANS is expressed during early embryonic development in zebrafish, with two duplicated genes for *NANS (NANSa* and *NANSb*). Expression begins at 50% epiboly and at 14 to 19 somite stages in the myotome and pharyngeal arch skeleton. Knockdown of *NANSa* results in an abnormal and complex phenotype regarding skeletal development in the head region at 6 dpf, while no such phenotype is evident for *NANSb*. The abnormal effects of *NANSa* knockdown can be partially rescued by the exogenous addition of Neu5Ac. In human, bi-allelic mutations in the NANS gene have been linked to infantile developmental delay and skeletal dysplasia (van Karnebeek *et al.*, 2016).

Two additional enzymes involved in sialylation also influence cartilage formation and bone development. In zebrafish, CRISPR/Cas9 deletion of *NEU1* results in abnormal embryogenesis with pleural effusion but not lethality. Neu1-KO fish display reduced body size, muscle atrophy, and vertebral curvature after 8 months, along with decreased expression of bone remodeling genes (runx2a, runx2b, mmp9). These traits closely resemble those seen in Neu1-KO mice and human sialidosis patients (Okada *et al.*, 2020). In mice, knockout of *MGAT2* results in increased osteogenic activity just 7 days after birth (Wang *et al.*, 2001).

The Bone SialoProtein (BSP) is a highly phosphorylated and glycosylated secretory protein enriched in Sia, essential for forming the extracellular matrix and ensuring complete mineralization of bones in mice. Removal of terminal sialylation of BSP triggers the downregulation of osteogenesis-related osteocalcin, osteoprotegerin, and vitamin D receptor at the mRNA and protein levels (Malaval *et al.*, 2008; Xu *et al.*, 2017).

Muscles

Sialome imaging in live zebrafish embryos using copper-free click chemistry revealed that sialic acids are present in muscle cells, in addition to ectodermal-derivatives at 24 hpf (Hong *et al.*, 2019). At 30 and 48 hpf, a dense sialylation is observed in intermyotomal boundaries, and at the surface of muscle fiber cells within the myotomes, known to express ST8Sia3 (Agarwal *et al.*, 2015). *ST8Sia3* is initially expressed in somites at 20 hpf. Morpholino-knockdown of *ST8Sia3* leads to anomalies in somite morphology, including defects in the segment boundary and the myotendinous junction at 35 hpf (Bentrop *et al.*, 2008). The phenotypes might either be somitic or secondary due to axonal defects (Flanagan-Steet and Steet, 2013). The *ST6Gal2* gene, which is expressed since the gastrula stage, is found in the mesoderm of the prechordal plate, in early somite, at the borders of the neural plate, with persistent expression in the last formed somite at 24 hpf (Petit *et al.*, 2010). The inactivation

of the zebrafish *MGAT1* gene, alongside the disruption of complex N-glycans results in skeletal and cardiac muscle defects at 28 and 58 hpf (Hall *et al.*, 2023). Gene targeting experiments using morpholinos against *NAL* generate skeletal myopathy and cardiac edema at 48hpf, mimicking a human disease phenotype (Wen *et al.*, 2018). Additionally, zebrafish with *NEU1* CRISPR/Cas9 deletions exhibit muscle atrophy and reduction in muscle determinants after 8 months (Okada *et al.*, 2020). *NEU3.2*, analogous to human *NEU2*, is essential for zebrafish skeletal muscle differentiation; morpholino knockdown leads to major embryonic defects in somites, the heart, and anterior-posterior axis formation (Zizioli *et al.*, 2023). Furthermore, knockdown in zebrafish using morpholino oligonucleotides targeting the sialomucin *endolyn (CD164)* results in pericardial edema at 48 hpf (Mo *et al.*, 2012).

In medaka embryos, the knockout of *O-sulfotransferase Wscd1,* responsible for transferring the sulfonyl group to the hydroxy group Sia, caused heart arrhythmias, decreased ventricular contractile force, and reduced levels of cardiac myosin heavy chain at 8 dpf (Ertunc *et al.*, 2022). The medaka *ST6Gal1* knockouts demonstrated pronounced cardiac abnormalities between 7 and 16 days post-fertilization (dpf), resulting in mortality at 14–18 dpf. This lethality was reversed by expression of ST6Gal1, ST6Gal2, and ST3Gal4, indicating that sialylation, rather than a specific sialic acid linkage, is critical for proper cardiac development (Omoto *et al.*, 2023).

In zebrafish, the depletion of *GNE* impairs skeletal muscles, leading to several abnormalities (Livne *et al.,* 2012; Daya *et al.,* 2014). In mice, the disruption of sialic acid synthesis results in embryonic lethality at 8.5 dpf, accompanied by poor differentiation of skeletal and cardiac muscle cells, and nerve cells (Varki, 2017). Homozygous inactivation of the *GNE* gene in the UDP-GlcNAc 2-epimerase domain causes early embryonic lethality in mice between 8.5 and 9.5 dpf due to a loss of *de novo* biosynthesis that cannot be compensated by a contribution of maternal sialylated glycoproteins (Schwarzkopf *et al.*, 2002). Heterozygous *GNE*-deficient mice survived with reduced sialylation. GNE also plays a significant role in skeletal and cardiac muscle development from mouse ESC. Cardiac and muscle cells in *GNE -/-* develop at the same time as control cells, but cardiac cells degrade rapidly with decreased beating capacity, and skeletal muscle cells are rare (Milman Krentsis *et al.*, 2011). Mutations in the *GNE* gene are linked to a rare and unique recessive human neuromuscular disorder known as hereditary inclusion body myopathy (HIBM) (Eisenberg *et al.*, 2001; Carrillo *et al*., 2018), and to sialuria (Leroy *et al.*, 2001).

In mouse myoblasts C2C12 cell line, differentiation depends on a decrease in α2,6-linked Sia. The suppression of ST6Gal1 impacts Notch signaling pathway activity, down-regulates Pax7 expression, triggers earlier myotube fusion, and the suppression of *ST6Gal1* (Vergé *et al.*, 2020).

Kidneys

The urinary system in amniotes originates from the intermediate mesoderm. In lower vertebrates, such as fish, the mesonephros is the final kidney. Endolyn (CD164), which associates with the chemokine receptor CXCR4, is expressed in the proximal tubule and distal segments of the kidney. In zebrafish, it is expressed in the developing pronephros. Targeting *sialylated endolyn* with morpholino oligonucleotides leads to hydrocephaly, body curvature, altered clearance of the pronephric kidney, in addition to pericardial edema at 48 hpf (Mo *et al.*, 2012).

In mammals, the metanephros is the final kidney (with nephrogenesis starting at 11 dpf in mice and 5 weeks in humans (Desgrange and Cereghini, 2015). Studies in rodents reported that the high sialylation of podocalyxin plays a pivotal role in maintaining the specialized epithelial podocyte architecture and facilitating glomerular filtration (Orlando *et al.*, 2001). Deficits in sialylation impair podocyte maturation (Weinhold *et al.*, 2012). Human podocalyxin has a mucin domain with a serine/threonine content for putative *O*-glycosylation modifications in its extracellular domain and also has five potential *N*-linked glycosylation sites and three putative glycosaminoglycan sites. Deficits in sialylation impair podocyte maturation and glomerular filtration in the metanephros (Li and Ding, 2019). In homozygous mutated (M712T *GNE*/*MNK* mutation) *GNE* mice, at 17 dpf, the embryos appear normal externally but die within 1 to 3 days after birth, after showing significant muscle deficiencies, severe glomerular hematuria, podocytopathy associated with hyposialylation of podocalyxin, and increased GNE epimerase activity. Interestingly, these phenotypes are rescued through dietary supplementation with ManNAc, a precursor of sialic acid that crosses cellular membranes (Galeano *et al.*, 2007; Quaggin, 2007), suggesting a potential method to mitigate some glomerulopathies associated with hyposialylation (Kakani *et al.*, 2012). Additionally, *CMAS* knockdown in mice yields mature kidney glomeruli of the inner cortex with enlarged glomerular urinary space one day after birth (Weinhold *et al.*, 2012). These mice exhibit impaired podocyte formation, foot process effacement, and decreased sialylation of nephrin and podocalyxin mirroring features found in patients with *nephrin* mutations related to Finnish-type congenital nephrotic syndrome.

Klotho, a glycosidase that removes α2,6-linked Sia, modulates the activity of the TRPV5 (transient receptor potential vanilloid type 5) calcium channel to allow transcellular calcium transport in the kidney distal tubule and may be relevant in kidney organogenesis (Cha *et al.*, 2008; Leunissen *et al.*, 2013).

Vasculature

Endothelial cells and angiogenesis

Endothelial precursors develop from splanchnopleural mesoderm into blood vessels, forming a primary capillary plexus that expands into a network (Jain, 2003). The recruitment of pericytes and smooth muscle cells contributes to this network formation (Carmeliet, 2005). This multistep process is regulated by factors such as the vascular endothelial growth factor (VEGF) and its receptors (Coultas *et al.*, 2005). VEGF stimulates angiogenesis (Simons *et al.*, 2016) through its binding to VEGFR2 carrying a2,6-sialylated glycans, *via* a reinforced electrostatic interaction (Chiodelli *et al.*, 2017; D'Addio *et al.*, 2020; Koch and Claesson-Welsh, 2012). In human umbilical cells (HUVEC), silencing of *ST6Gal1* results in ST3Gal1 upregulation and α2,3-sialylation of VEGFR2 that is still able to bind the VEGF ligand. The concomitant silencing of both *ST6Gal1* (affecting *N*-glycans) and *ST3Gal1* (affecting *O*-glycans) abolishes VEGF/VEGFR2 signaling, suggesting that only negatively charged sialic acids, but not their chemical presentation, are required to support VEGFR2 activation (Chiodelli *et al.*, 2017; D'Addio *et al.*, 2020). Losses of 9-*O*-acetylation of sialic acids are observed, with ST6Gal1 deficiency causing loss on the endothelium (Martin *et al*., 2002). Overexpression of *NEU1* blocks endothelial tube formation, while the overexpression of *ST6Gal1* restores it in human pulmo-

nary microvascular and artery endothelial cells (HPMEC, HPAEC) (Lee *et al.*, 2014). α2,6-sialylation also regulates VEGF receptors binding to PECAM1 and promotes endothelial cell migration and angiogenesis sprouting in mice (Imamaki *et al.*, 2018).

A recent study using a *GNE* loss-of-function mutation related to the *de novo* biosynthesis of Sia, showed *GNE* to be important for angiogenesis. A mouse model carrying one of these mutations (P735R in the *MNK* domain) was also detected in a pediatric macrothrombocytopenia, exhibiting defective angiogenesis and abnormal megakaryocyte accumulation combined with cerebral hemorrhages at 11 dpf and death at 12.5 dpf (Huang *et al.*, 2024).

Inactivation of *MGAT1* in mice results in lethality at 9.5 dpf with embryos displaying vasculature defects, in addition to neural tube anomalies and a *situs inversus* (Ioffe *et al.*, 1997).

The anti-adhesive sialomucin CD34 is involved in lumen formation. It is localized at endothelial contact sites, where it interacts to the underlying actin cytoskeleton to enable cell shape modification and lumen formation. The negatively charged (due to sialic acids) extracellular domains of CD34 create a narrow space, forming a slit through repulsive interactions between opposing surfaces (Robbins and Beitel, 2010; Strilić *et al.*, 2010).

The core 1 β1-3-galactosyltransferase (C1Galt1 or T-synthase) transfers Gal from UDP-Gal to GalNAc-Ser/Thr (Tn antigen) to form the core 1 *O*-glycan Galβ1-3GalNAc-Ser/Thr (T antigen), which serves as a precursor for extended and branched *O*-glycans necessary for angiogenesis and prevention of fatal embryonic brain hemorrhage. In wild-type mice, Neu5Acα2-3Galβ1-3GalNAc-Ser/Thr is localized in endothelial and hematopoietic cells during development. Gene-targeted mice lacking *T-synthase*, which cannot express the sialylated form of Tn antigen, manifest fatal brain hemorrhage at 14 dpf with a chaotic microvascular network in the brain, distorted capillary lumens, and abnormal association of endothelial cells with pericytes (Xia *et al.*, 2004) necessary structures to form the blood-brain barrier (Bauer *et al.*, 2014).

Interestingly, genetic disruption of Notch signaling in mice halts embryonic vascular expansion between 7.5 to 11.5 dpf and is lethal (Hofmann and Iruela-Arispe, 2007). Notch activity is modulated by the β 3-*N*-acetylglucosaminyltransferase radical Fringe which results in a GlcNAcβ1-3Fucose disaccharide that can be further elongated to form a sialylated tetrasaccharide, Siaα2-6Galβ1-4GlcNAcβ1-3Fuc (Moloney *et al*., 2000a; Moloney *et al*., 2000b).

Lymphangiogenesis

After their formation, lymphatic endothelium and lymph nodes in mice express LYVE1 at 9.5 days post-fertilization (Alitalo *et al*., 2005; Oliver and Alitalo, 2005), as do arterial and venous endothelium (Gordon *et al.*, 2008), and undergo a regulation by sialylation on LYVE1 (Nightingale *et al.*, 2009; Schauer, 2009).

Yolk sac endothelium and hematopoiesis in placental species

In placental species, an extraembryonic mesoderm (ExM) develops around the time of gastrulation as a supportive structure (Ross and Boroviak, 2020). In human, it contributes to the formation of the placental chorionic villi which infiltrate the endometrium, connect to the maternal circulation (Pham *et al.*, 2022), and form the extraembryonic vessels in the trophoblast villi.

PSA formed onto *N*-linked carbohydrate structures by ST8Sia4 are involved in the migration and invasion of human cytotrophoblasts (Hromatka *et al.*, 2013). During early erythropoiesis, PSA are also present on human hematopoietic progenitors and myeloid cells. Studies on *st8sia4* knockdown mice demonstrated their importance in the development of hematopoietic progenitors (Drake *et al.*, 2008; Drake *et al.*, 2009).

Sialomucin CD34, expressed on endothelial cells of the yolk sac, enhances trafficking and migration of hematopoietic cells by favoring their binding to E and P selectin on endothelial cells in humans. The lack of CD34 significantly impairs the expansion of hematopoietic progenitor cells *in vitro* (AbuSamra *et al.*, 2017).

Highly sialylated erythropoietin, produced by the yolk sac around 9 dpf, is crucial for early erythropoiesis following heartbeat activation in mice (Hirano and Suzuki, 2019). Due to its lower receptor affinity and extended serum half-life, highly sialylated erythropoietin more effectively stimulates erythrocyte production (Gross and Lodish, 2006).

Conclusion

Mesodermal cells, which are specified during gastrulation, develop into a broad range of cell types, including those forming the musculoskeletal system, kidneys and the cardiovascular system. The formation of mesoderm is guided by inductive processes that integrate both external factors, such as maternal and environmental influences, and internal self-organizing signals responsible for patterning and morphogenesis. Despite progress, it remains challenging to consistently understand how different mesodermal cell types emerge across various contexts and timeframes. Although the physiological roles of certain sialic acids (in particular, some biosynthetic enzymes) in the embryo have been identified, the exact functions of sialyltransferases remain poorly understood. Ongoing research continues to address the biological impact and mechanistic details of sialic acids, as well as how sialic acid glycoproteins operate. In many cases, gene knockouts affect multiple steps in organ formation, probably because the enzymes involved in sialylation often have overlapping functions with possible compensation. Additionally, clarifying discrepancies between species regarding embryogenesis timing and modalities is necessary. Recent advancements in single-cell 'omics' have facilitated the reconstruction of potential developmental trajectories and dynamics of lineage specification at the cellular level (Ferretti and Hadjantonakis, 2019). The emergence of gastruloids -3D aggregates capable of self-organization and mimicking aspects of gastrulation - holds promise to study these mechanisms beyond the confines of embryos from placental species (Morales *et al.*, 2021; Simpson and Alberio, 2023). Furthermore, organoid systems that replicate organ structures or organs-on-chips derived from stem cells with vascularization capabilities, such as those for the kidney, provide models to enhance our understanding of mammalian organogenesis (Liu *et al.*, 2024; Ryan and Cleaver, 2022). Moreover, a complete model of a human embryo created 14 days after implantation from embryonic stem cells may offer a simpler way to explore early stages of development in accordance with ethical laws (Oldak *et al.*, 2023). All these innovative biological systems could help clarify specific aspects of developmental processes from early and late organogenesis in mesoderm-derived tissues, as well as the precise role of sialylated effectors and enzymes involved in sialylation.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: I.F., A.H.L and K.C. Writing: I.F., P.D., A.F., M.M., A.M., Y.U., N.Y., C.M., J.F.B., A.H.L. and K.C.

Funding

This work was supported by the CNRS and the University of Lille.

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