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Multilineage coordination mechanisms in craniofacial organ development

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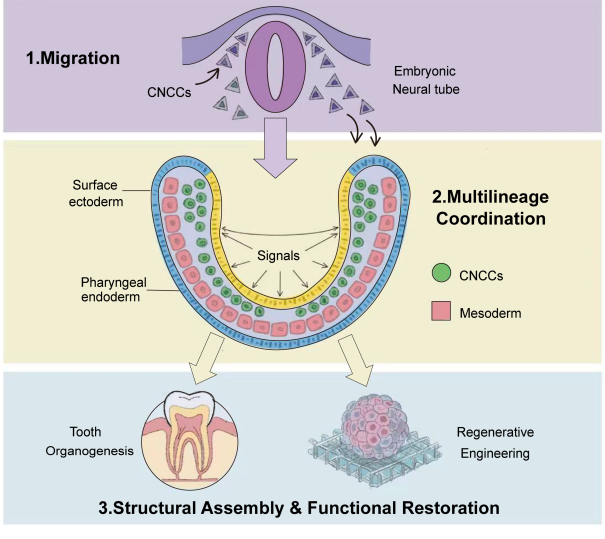
ABSTRACT: Craniofacial development is a complex process shaped by the coordinated work of multiple embryonic lineages, including cranial neural crest cells (CNCCs), surface ectoderm, mesoderm, and pharyngeal endoderm. CNCCs make major contributions to craniofacial skeletal and connective tissues, but craniofacial morphogenesis cannot be explained by one lineage alone. This process depends on active interactions among different cell lineage populations, which provide both structural components and regulatory signals. Recent advances in single-cell and spatial omics technologies have shown that lineage specification and differentiation are controlled by intrinsic gene regulatory programs and extrinsic cues, including morphogen signaling, extracellular matrix remodeling, and biomechanical forces. These interactions are especially critical during neural crest migration, epithelial-mesenchymal communication, and organogenesis of craniofacial structures, including teeth and glands. In this review, we summarize how multiple lineages contribute to craniofacial formation and highlight the coordinated mechanisms that bring these lineages together during development. We combine classical embryological concepts with recent molecular findings to show that craniofacial morphogenesis is a systems-level process. This view helps explain craniofacial disorders and supports regenerative strategies that recreate complex tissue architecture and function.



KEYWORDS: multilineage, coordination, craniofacial organ, development

1 Introduction

Craniofacial development depends on close coordination among several embryonic lineages, including cranial neural crest cells (CNCCs), surface ectoderm, mesoderm, and pharyngeal endoderm. CNCCs arise from the neural plate border. These cells then migrate over long distances and form much of the craniofacial skeleton and connective tissues. At the same time, the surface ectoderm forms epithelial structures, including the epidermis and oral epithelium. The mesoderm contributes to the formation of muscles and blood vessels. The pharyngeal endoderm gives rise to epithelial linings and glandular components. These lineages work together and build a combined structure that shapes craniofacial anatomy[1].

Craniofacial morphogenesis is shaped not only by the separate contributions of each cell lineage, but also depends on coordinated interactions among these cell populations. Signals emanating from adjacent tissues orchestrate neural crest induction, migration and differentiation. Such tissues include ectodermal signaling centers, mesoderm-derived endothelium, and endodermal niches[2]. Epithelial–mesenchymal interactions also guide these events, while neurovascular and musculoskeletal coupling sustains appropriate tissue patterning and functional integration[3]. These reciprocal communications engage canonical signaling pathways as well as biomechanical cues[4]. Collectively, these findings demonstrate that

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craniofacial development is governed by multiple interconnected regulatory tiers.

Understanding this multilineage coordination is important for both developmental biology and regenerative medicine. In regenerative medicine, researchers still face a major challenge when they try to regenerate craniofacial tissues with the right shape and function[5]. This goal requires more than knowledge of individual cell types. It also requires a clear understanding of how different lineages interact during development to build complex structures[6]. These mechanisms may guide tissue engineering and personalized regenerative therapies. They may also help researchers recreate tissue architectures that are closer to native development.

In recent years, single-cell and spatial omics technologies have changed how researchers study craniofacial development. These methods provide high-resolution maps of lineage trajectories, gene regulatory networks, and intercellular communication. These maps help researchers identify cell heterogeneity and dynamic interactions that were not clearly recognized before. With these advances, the field can now move beyond mainly descriptive models. Researchers can develop a more integrated understanding of craniofacial morphogenesis[7].

In this review, we bring together current knowledge about the evolution and coordinated mechanisms of multiple cell lineages in craniofacial construction. We combine classical embryological insights with new molecular and systems-level data. This approach helps us show how lineage diversification and intercellular coordination work together to shape craniofacial development. We also discuss how these findings may guide future research and therapeutic applications.

2 Multilineage contributions to craniofacial organs

In embryonic development, the face arises from coordinated contributions of neural ectoderm-derived CNCCs, surface ectoderm, pharyngeal endoderm, and cranial mesoderm[8]. First, neural-ectoderm contributes to much of the craniofacial mesenchyme that later differentiates into cartilage, bone, cranial neurons/glia, and connective tissues[9]. Surface ectoderm contributes to the epidermis and to numerous craniofacial epithelial derivatives, including enamel-forming ameloblasts, anterior tongue taste buds, and the epithelial components of some salivary glands, such as the parotid gland[10]. Mesoderm contributes the musculature and vasculature that integrate with the neural crest-derived skeletal framework[11]. Therefore, the craniofacial complex is a multilineage composite: its final anatomy requires crest-derived skeletal and connective tissues to assemble in register with ectodermal epithelia and mesoderm-derived myogenic and endothelial populations, within an endoderm-partitioned pharyngeal scaffold[12,13]. Next, we will elaborate in detail on the respective contributions of the multi-lineage origins (neural crest, ectoderm, and mesoderm) to craniofacial development at different stages.

2.1 The craniofacial development process

2.1.1 Neural crest induction and migration stage

During craniofacial development, the first developmental transition is "neural tube to neural crest transition". This process is not only a change of location but a change of competence, generated at a tissue interface.

In Gilbert's synthesis of classical experiments, juxtaposition of prospective neural plate with prospective epidermis is sufficient to induce neural crest formation, and both domains contribute to the emerging neural crest population, showing that induction is an inter-tissue event instead of an independent neural tube program[14]. After induction, the cranial crest's defining feature is extensive migration coupled to fate diversification: cranial crest streams provide craniofacial mesenchyme and later contribute to cartilage and bone of the face and neck, as well as pigment and cranial nerves, while other crest derivatives across the body include peripheral neurons/glia and melanocytes. Importantly, "where they go" strongly predicts "what they become," because the fate of neural crest cells (NCCs) depends to a large degree on where they migrate and settle[15]. This conditionality sets up a core logic for craniofacial development: pattern is encoded both intrinsically (e.g., positional identity carried by crest cells) and extrinsically (signals received en route and within target tissues). Thus, craniofacial form arises from the convergence of lineage history and local tissue interactions[16]. When this coordination is disturbed during induction, migration, or differentiation, craniofacial anomalies may occur. These anomalies include clefts and syndromic malformations. Modern clinical-oriented reviews of neural crest biology also emphasize this link[17] (Fig. 1a-c).

2.1.2 Pharyngeal arches and facial prominences

The transition from neural crest to pharyngeal arches shows how multiple lineages are arranged in space within one developmental unit. The pharyngeal arches are paired bulges around the anterior foregut. Each arch forms a combined epithelial–mesenchymal structure. The ectoderm covers the outside of the arch, and the endoderm lines the inside. The mesenchymal compartment fills the arch, where neural crest–derived mesenchyme surrounds a central group of mesodermal cells[18]. This arrangement is closely related to later lineage-specific outcomes. In Graham's overview, the ectoderm forms the epidermal covering and sensory neurons of epibranchial ganglia. The endoderm forms epithelial lining and endocrine glands. The neural crest forms connective and skeletal tissues, while the mesoderm forms musculature and endothelial cells of arch arteries[19].

The third transition—"arches to facial prominences and fusion"—shows how multilineage assembly is translated into the recognizable anatomy of nose, jaws, and palate. Reviews of craniofacial morphogenesis describe the vertebrate face as a set of outgrowths (prominences) that must be positioned, expanded, and then merged or fused in a temporally ordered way. The embryonic face includes seven prominences, including the frontonasal process (FNP) and paired lateral nasal, maxillary, and mandibular prominences, each colonized by CNCCs that carry distinct molecular signatures and interact with different epithelia during development[20]. Proper formation of the FNP requires interactions between neural crest and both forebrain epithelium and facial ectoderm, showing again that crest-derived mesenchyme depends on epithelial partners to realize region-specific morphogenesis[21]. The maxillary and mandibular prominences—derived from the first arch—require coordinated interactions among surface ectoderm, neural crest, mesoderm, and pharyngeal endoderm. This makes their development an explicit example of multilineage integration rather than single-lineage construction[22]. Roth et al. also describe facial development as a coordinated process involving the establishment of the pharyngeal arches and facial prominences.

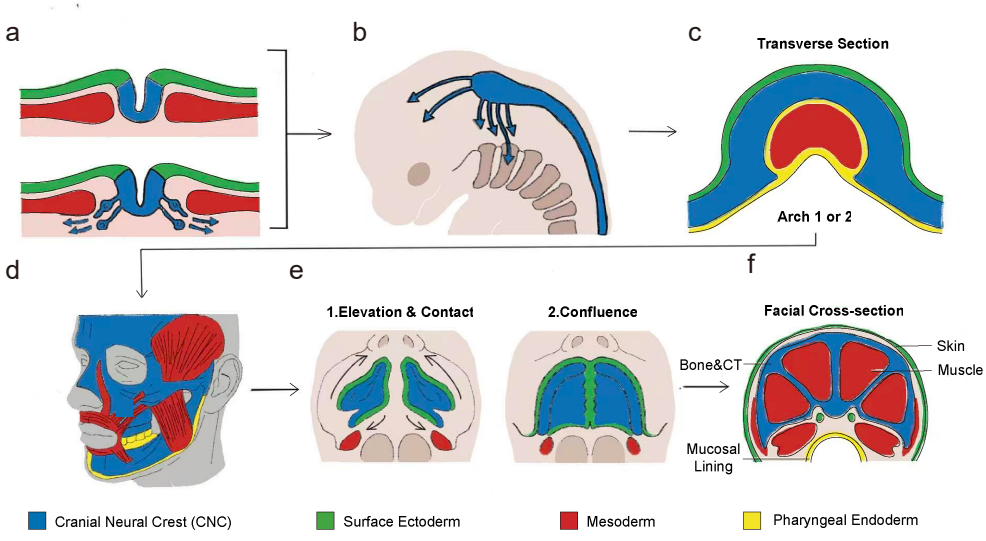

Figure 1 Multilineage coordination during craniofacial development from neural crest induction to facial morphogenesis. (a) At the neural tube border, cranial neural crest cells (CNCCs) arise at the interface between neural ectoderm and surface ectoderm. After induction, CNCCs undergo delamination and epithelial-to-mesenchymal transition. These changes allow CNCCs to gain migratory competence. (b) CNCCs migrate in well-defined streams into the frontonasal region and pharyngeal arches. These migration paths strongly affect their later cell fate. Along these routes, CNCCs contribute to craniofacial mesenchyme and give rise to different derivatives, including cartilage, bone, and neural tissues. (c) In the pharyngeal arches, multiple lineages come together to form a coordinated epithelial–mesenchymal structure. Each arch contains an outer ectodermal layer, an inner endodermal lining, and a mesenchymal core. In this core, neural crest–derived cells surround a central mesodermal population. This arrangement forms the characteristic "crest shell–mesoderm core" organization. (d) Facial prominences include the frontonasal, maxillary, mandibular, and lateral nasal processes. These structures form as neural crest-derived mesenchyme proliferates and becomes organized in space under the guidance of surrounding epithelia. Their growth and position depend on coordinated interactions among the neural crest, ectoderm, mesoderm, and endoderm. (e) Palatal development shows how different lineages coordinate during morphogenesis. Palatal shelves elevate and fuse through the formation of an epithelial seam. The epithelial seam then breaks down, and this process allows mesenchymal continuity to form. Disruption of this process can lead to cleft phenotypes. (f) Final craniofacial structures form through the integration of multiple lineages. Neural crest-derived skeletal and connective tissues align with ectoderm-derived epithelium, mesoderm-derived musculature and vasculature, and endoderm-derived pharyngeal lining.

Extensive NCC proliferation and migration drive the outgrowth of these prominences, which subsequently converge to form the nose and upper and lower jaws[20]. The palate provides a clear example of this process. Palatal shelves need to elevate, grow, and fuse during development. Neural crest-derived mesenchyme and ectoderm-derived epithelium both contribute to this process. Palatal fusion requires the formation and later breakdown of an epithelial seam, which allows mesenchymal continuity to form[23]. In this view, "fusion" is not only a final shape change. It is a lineage-coordinated process. Epithelial behavior from the ectoderm and mesenchymal proliferation and organization, which are largely neural crest-derived within the arch context, need to work closely together to prevent clefting phenotypes[20] (Fig. 1d-e).

2.2 Multilineage contributions

2.2.1 Neural crest cells

NCCs are now understood as a population that varies over time and space, rather than as one uniform stem-cell pool. These cells arise step by step from neural plate border progenitors. The progenitors first gain border identity, and they later become premigratory NCC progenitors at the dorsal neural tube. Following epithelial-to-mesenchymal transition and delamination, these cells enter an early migratory state in which many retain broad multipotency, although single-cell and lineage-tracing studies indicate that fate bias can emerge surprisingly early and varies across axial levels[24].

NCCs are a vertebrate-specific population of highly migratory cells that arise from the dorsal region of the developing neural tube[25]. The developmental hierarchy proceeds from neural plate border progenitors to premigratory NCCs, then to migratory multipotent NCCs, and later to step by step fate-restricted progenitors, including neuroglial progenitors that produce sensory, sympathetic and parasympathetic neurons as well as peripheral glia; melanocyte-biased progenitors; and, particularly in the cranial region, ectomesenchymal progenitors that form cartilage, bone, connective tissue and smooth muscle[26]. Importantly, lineage progression is not strictly linear for all derivatives: Schwann cell precursors, which arise from NCC-derived peripheral glial lineages along developing nerves, act as a secondary progenitor state and can still produce multiple "late" derivatives, including parasympathetic neurons, chromaffin cells, melanocytes and mesenchymal cell types in specific contexts[27]. After their induction, these cells undergo delamination and migrate widely throughout the embryo, where they form a diverse range of derivatives, including peripheral and enteric neurons, glial cells, melanocytes, and smooth muscle cells[28]. In the cranial region, NCCs make major contributions to the formation of the cartilage and bone of the skull, as well as the facial and pharyngeal skeleton[29]. More specifically, rostral cranial NCCs largely produce the frontonasal skeleton and the membranous bones of the skull. In contrast, posterior cranial NCCs populate the pharyngeal arches (PAs) and contribute to structures such as the jaw, middle ear, hyoid, and thyroid cartilages[30].

2.2.2 Ectoderm

During craniofacial development, the surface ectoderm is a major

embryonic germ layer that forms multiple epithelial derivatives essential for facial structure and function, including the epidermis of the skin, oral epithelium, and ectodermal appendages such as teeth and salivary glands[31]. In the oral cavity, ectoderm-derived epithelium undergoes precise spatial patterning to produce distinct organs, including the dental epithelium that later forms enamel through reciprocal epithelial–mesenchymal interactions[32], as well as taste buds and salivary glands arising from patterned oral epithelial fields[33].

The ectoderm, the outermost germ layer of the vertebrate embryo, is step by step regionalized into a series of fate-restricted progenitor populations through coordinated patterning by BMP, WNT, FGF and retinoic acid signals[34]. At the earliest stage, pluripotent epiblast cells form ectodermal progenitors. This are then subdivided during gastrulation into non-neural ectoderm and neural ectoderm. Neural ectoderm progenitors form the neural plate and later differentiate into neuroepithelial and radial glial progenitors that generate the central nervous system, including neurons and glial cells of the brain and spinal cord. In parallel, non-neural ectoderm produces surface ectoderm progenitors. This further form the epidermis and its appendages, such as keratinocytes, hair follicles, sebaceous glands and components of sensory epithelia[34]. Importantly, a specialized population arises at the border between neural and non-neural ectoderm, termed the neural plate border, which contains multipotent border progenitors that later segregate into neural crest progenitors and cranial placode progenitors. Neural crest progenitors undergo epithelial-to-mesenchymal transition and generate diverse derivatives including peripheral neurons, glia, melanocytes, craniofacial cartilage and bone, and smooth muscle. In contrast, placodal progenitors form sensory structures such as the lens, olfactory epithelium, inner ear, and cranial sensory ganglia[35]. Thus, ectodermal development proceeds in a hierarchical manner from broad ectodermal progenitors to region-specific intermediate progenitors and finally to highly specialized lineages, with progressive restriction of developmental potential coupled to in space and time regulated signaling and transcriptional control[36].

2.2.3 *Mesoderm*

Mesodermal progenitors are specified during gastrulation, when epiblast cells ingress through the primitive streak and are progressively allocated into distinct mesodermal domains according to their spatial position, timing of ingression, and signaling environment[2]. Among these domains, the cranial paraxial mesoderm and pharyngeal arch mesoderm make major contributions to craniofacial development. Mesoderm-derived cells primarily give rise to craniofacial skeletal muscles and vascular endothelium, while also contributing to selected connective tissues, the cranial base, and parts of the cranial vault[37]. Thus, mesodermal fate determination in the head involves progressive restriction of early mesodermal progenitors toward region-specific cranial mesodermal populations and subsequently toward myogenic, vascular, and skeletal lineages[38].

The fate of cranial mesoderm is also closely linked to its spatial relationship with cranial neural crest cells (CNCCs). Lineage-mapping studies in mouse embryos showed that cranial paraxial mesoderm and CNCCs migrate into adjacent or overlapping regions and intermingle extensively within periocular, facial, periotic, and cervical mesenchyme[39]. Within the pharyngeal arches, however, these two populations display a more organized spatial arrangement: CNCC-derived mesenchyme occupies the peripheral region and surrounds a central mesodermal core[39]. This "crest shell–mesoderm core" organization is consistent with the general architecture of the pharyngeal arches, in which neural crest-derived mesenchyme surrounds centrally located mesoderm beneath the ectodermal and endodermal epithelia[40]. Such spatial organization provides an anatomical basis for coordinating neural crest-derived skeletal patterning with mesoderm-derived myogenesis. Consequently, mesodermal differentiation is not an isolated lineage program but is shaped within a multilineage environment in which neural crest, mesoderm, and neighboring epithelia jointly coordinate craniofacial tissue patterning and morphogenesis[12].

Finally, mesodermal contributions extend beyond muscle to the vasculature, adding another dimension to multilineage integration that is often overlooked in crest-centric narratives. Noden and Francis-West note that endothelial precursors (angioblasts) are ubiquitous throughout paraxial mesoderm and are highly motile, penetrating crest cell populations while the latter are en route to branchial and periocular sites, enabling rapid vascularization of peripheral mesenchymal tissues[12]. This description shows a clear developmental route by which mesoderm-derived vasculature can grow into migrating crest-derived mesenchyme. In this way, new facial tissues can receive blood supply as they expand and differentiate[41]. Graham also describes musculature and endothelial cells of arch arteries as mesoderm-derived tissues, while the neural crest forms connective and skeletal tissues. This view suggests that vascular patterning and skeletal patterning need to be matched within the lineage mosaic of each arch[19]. Together, these studies support the view that craniofacial morphogenesis cannot be explained by a single lineage path. It is a coordinated assembly process. In this process, CNCC-derived skeletal and connective frameworks, mesoderm-derived myogenic and endothelial programs, and ectoderm/endoderm-derived epithelial boundaries need to meet at the right time and in the right place. This coordination also helps explain why craniofacial development is both stable and sensitive to disruption. It is stable because vertebrates often use conserved interactions among multiple tissues. It is sensitive because changes in signaling networks or cell–cell coordination can spread through the system and lead to clefts, skeletal dysmorphology, or neuromuscular mismatch[42]. In sum, the craniofacial complex can be described as a multi-lineage composite. This is not only because its tissues come from the neural crest, ectoderm, endoderm, and mesoderm. It is also because its normal form depends on the coordinated integration of these lineages. This integration occurs during several linked steps, from neural tube border induction to arch colonization, and from prominence growth to fusion. During these steps, mesodermal muscle and vasculature continue to enter and connect with crest-structured facial mesenchyme[43](Fig. 1f).

The above studies show that multiple lineages contribute to craniofacial organ development, including surface ectoderm, mesoderm, and neural crest. Next, we will focus on how those different-origin lineages are coordinated in the craniofacial development process.

3 Multilineage coordination mechanisms in craniofacial organ development

Craniofacial development requires different cell lineages to develop in the correct spatial and temporal relationship. CNCC-derived

skeletal and connective tissues must be coordinated with ectodermal and endodermal epithelia, mesoderm-derived muscle and vasculature, and the developing nervous system[44]. These tissues do not develop as separate units. Their growth, patterning, and differentiation influence neighboring cell populations throughout morphogenesis. A change in one lineage may therefore affect the organization or function of other tissues[45]. Multilineage coordination is important for linking lineage-specific differentiation with the final shape and function of craniofacial organs. The mechanisms underlying this coordination are discussed below.

3.1 Major modes of multilineage coordination

The following section discusses multilineage coordination according to major modes of interaction. These coordination modes include: A central mode involves epithelial–mesenchymal interactions, where ectodermal or endodermal epithelia control NCC behavior and guide organ morphogenesis.

Another key layer of coordination comes from biochemical signaling networks, including pathways such as WNT, BMP, FGF, SHH, TGFβ, and VEGF. This send positional cues and fate-specifying information between lineages. Multilineage coordination is also shaped by biomechanical and extracellular-matrix-mediated regulation, as cell adhesion, cytoskeletal remodeling, tissue stiffness, and muscle-generated forces collectively influence morphogenesis.

In addition, neurovascular and musculoskeletal niche interactions play essential roles, with endothelial cells, nerves, and muscles providing instructive support for epithelial, mesenchymal, and skeletal development.

The examples below are organized to illustrate how these interconnected coordination modes operate across different craniofacial tissues.

3.1.1 Epithelial–mesenchymal signaling regulates NCC EMT and migration

Ectodermal and mesodermal inputs are important not only during NCC induction. These inputs also guide delamination, epithelial-to-mesenchymal transition (EMT), and migration. After specification, NCCs need to weaken cell adhesion, reorganize cell polarity, regulate proliferation, and gain motility. These cells carry out these changes as they move through the space between non-neural ectoderm and surrounding mesoderm[20]. These steps are not random cellular changes but responses to external biochemical and biomechanical cues, including repulsive and attractive guidance signals, confinement, tissue fluidity, and mesodermal stiffening[46]. Antiguas et al. provide a mechanochemical framework for understanding how extracellular signaling, adhesion remodeling, and cytoskeletal dynamics are linked during NCC EMT and craniofacial morphogenesis[8]. In their review, NCCs are described as being specified at the border between the neural plate and non-neural ectoderm by WNT, BMP, and FGF signaling gradients together with a neural crest gene-regulatory network. After induction, these signals are coupled to EMT, during which NCCs weaken epithelial cell–cell adhesion by reducing E-cadherin-mediated adherens junctions. This loss of E-cadherin is not only a marker of EMT, but a functional step that loosens cadherin–catenin complexes connected to the actin cytoskeleton, therefore allowing cells to remodel junctions, change polarity, and acquire motility[47]. Adhesion remodeling is further linked to activation of small Rho GTPases. This control actin organization, protrusive activity, and actomyosin contractility[48]. At the same time, YAP represents a mechanosensitive effector that connects changes in cell shape, tension, and adhesion to transcriptional responses[49]. Thus, WNT/BMP/FGF signaling can be interpreted as establishing and stimulating NCC migratory competence[50]. In contrast, E-cadherin downregulation and Rho/YAP-dependent mechanotransduction execute the physical transition from an epithelial adhesive state to a migratory mesenchymal state. This model links soluble morphogen signaling with cell–cell adhesion, cytoskeletal remodeling, and mechanical control, explaining how NCC delamination and migration contribute to coordinated facial and palatal morphogenesis[8].

Among the external regulators of cranial NCCs, the surface ectoderm appears especially important as an instructive signaling center. Van Otterloo et al. demonstrate that the facial surface ectoderm is not merely a covering layer over NCC-derived mesenchyme, but an active tissue that controls the growth, patterning, and morphogenesis of the underlying cranial neural crest populations. In their mouse study, loss of AP-2α and AP-2β in early embryonic ectoderm caused severe craniofacial abnormalities, including clefts of the upper face and mandible as well as abnormal jaw fusion. Importantly, the ectodermal defect altered chromatin accessibility and gene expression within ectodermal lineages and was associated with reduced WNT pathway activity. At the same time, ectodermal WNT1 overexpression rescued the clefting phenotype. These results strongly support a model in which transcriptional programs in the ectoderm shape the signaling environment received by cranial NCCs[51](Fig. 2a).

3.1.2 Mesoderm-derived endothelium regulates NCC morphogenesis

An equally important extrinsic regulator is the mesodermal and endothelial environment. This influences NCC survival, migration, and differentiation through both structural and signaling mechanisms. Milgrom-Hoffman et al. provide evidence that endothelial cells are essential for cranial neural crest morphogenesis during early embryogenesis. In mouse embryos lacking mesodermal Flk1, endothelial cells were absent, the vascular system was poorly formed, and the cardio-craniofacial field was severely deformed. Under these conditions, cranial NCC migration and survival were impaired, accompanied by reduced Tgfb1 expression and alterations in extracellular matrix (ECM) composition. The authors therefore propose that endothelial cells coordinate morphogenesis at least in part through a conserved Tgfb1-dependent ECM remodeling circuit. This is an important step because it moves the vasculature beyond its traditional nutritive role and identifies endothelial cells as organizers of the extracellular context in which NCCs migrate and assemble craniofacial structures[52].

Recent work further extends this vascular perspective from early morphogenesis to later lineage differentiation. Dash et al. examined endothelial-specific loss of *Med23* and found craniofacial defects including micrognathia and cleft palate, despite apparently normal neural crest formation and migration. The key defect lay in the osteogenic differentiation of NCC-derived craniofacial cells. This was severely impaired in mutant embryos. Spatial transcriptomics revealed downregulation of vascular and osteogenic genes, including *Vegfr1* and *Col1a1*, together with elevated HIF1α and reduced VEGF signaling, consistent with a hypoxia-associated block in osteoblast maturation. Rescue of craniofacial ossification by HIF1α inhibition combined with VEGFA supplementation strongly supports the conclusion that vascular patterning instructs

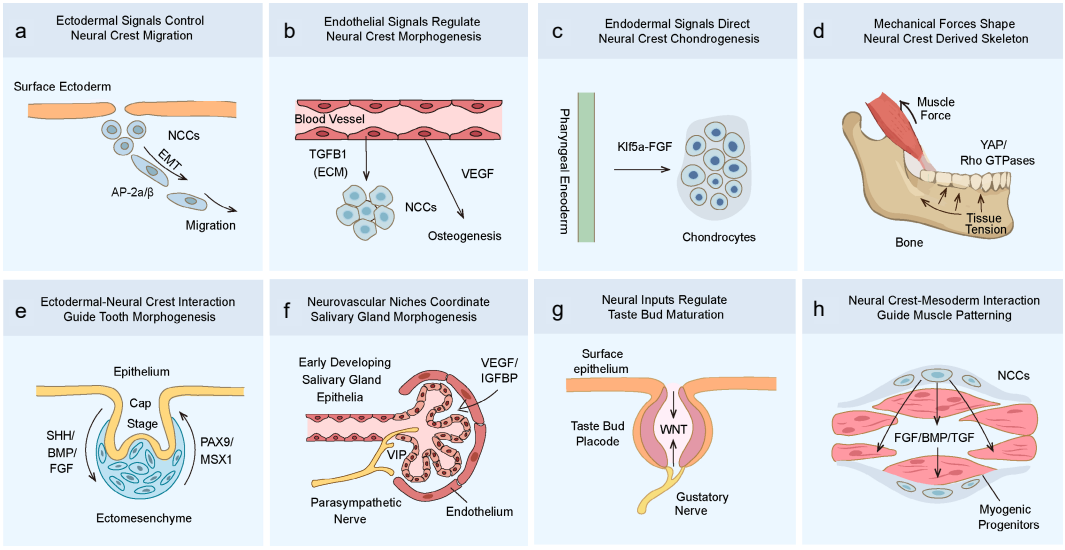

Figure 2 Coordinated tissue interactions and signaling mechanisms underlying craniofacial construction. (a) Ectodermal regulation of NCC EMT and migration. The surface ectoderm acts as an instructive signaling center that controls NCC EMT, migration, and transcriptional programs. Alterations in ectodermal transcription factors (e.g.,AP-2α/β) reduce WNT signaling, leading to impaired NCC behavior and craniofacial malformations such as clefting. (b) Mesoderm-derived endothelium regulates NCC morphogenesis and differentiation. Endothelial cells coordinate NCC survival, migration, and lineage progression through TGFβ1-dependent extracellular matrix (ECM) remodeling. Vascular defects result in hypoxia-associated signaling changes (e.g., HIF1α activation, reduced VEGF signaling), impairing osteogenic differentiation of NCC-derived mesenchyme. (c) Pharyngeal endoderm directs NCC-derived cartilage formation. Endodermal signaling, mediated in part by the Klf5a–FGF axis, regulates proliferation and differentiation of NCCs within the pharyngeal arches. Without proper endodermal cues, NCCs fail to complete normal chondrogenesis, even though they migrate to the correct region at the beginning. (d) Biomechanical and musculoskeletal inputs shape NCC-derived skeletal structures. Muscle attachment generates mechanical forces, and these forces influence craniofacial bone growth and morphology. Cytoskeletal remodeling and mechanotransduction pathways, including Rho GTPases and YAP signaling, help connect tissue tension with NCC-derived skeletal development. (e) Reciprocal ectoderm–NCC interactions guide tooth morphogenesis. Tooth development depends on dynamic epithelial–mesenchymal interactions.Oral epithelium initially provides instructive signals (e.g., SHH, BMP, FGF), followed by reciprocal signaling from NCC-derived ectomesenchyme (e.g., PAX9, MSX1), which regulates epithelial patterning, cusp formation, and differentiation. (f) Neural and vascular niches coordinate salivary gland epithelial morphogenesis. Parasympathetic innervation promotes ductal morphogenesis and lumen formation through VIP-dependent signaling. Endothelial cells regulate epithelial organization and progenitor maintenance through VEGF- and IGFBP-mediated pathways. (g) Epithelial-intrinsic programs and neural input work together during taste bud development. Early taste bud specification mainly depends on epithelial-intrinsic signaling, especially WNT/β-catenin activity. At later stages, gustatory innervation becomes essential for proper taste bud maturation and maintenance. (h) Neural crest–mesoderm interactions guide craniofacial muscle patterning. Cranial NCCs regulate mesoderm-derived myogenic progenitors through both structural support and signaling mechanisms.NCC-derived connective tissues and fibroblastic cells provide cues (e.g., FGF, BMP, TGFβ signaling) that control muscle proliferation, alignment, and differentiation, ensuring proper musculoskeletal integration.

intramembranous ossification in the face. The craniofacial vasculature therefore acts as a signaling partner that helps determine when and how NCC-derived mesenchyme matures into mineralized skeletal tissue[8] (Fig. 2b).

3.1.3 Endodermal–mesenchymal signaling directs NCC-derived cartilage formation

Signals from the pharyngeal endoderm represent another crucial axis of external control over neural crest-derived craniofacial structures. Li et al. focus on zebrafish pharyngeal cartilage, a classical neural crest derivative, and show that endodermal *klf5a* is necessary not for NCC specification or migration, but for their subsequent proliferation and differentiation within the pharyngeal region. *Klf5a* deficiency reduced *fgfbp2b* expression in pharyngeal endodermal pouches and caused defective cartilage morphogenesis. *klf5a* mRNA rescued these defects. However, *fgfbp2b* alone could not restore normal development. This result suggests that *klf5a* controls a broader endodermal signaling network, and FGF modulation is one important part of this network. This finding helps us understand craniofacial patterning more clearly. This finding shows that early NCC deployment and local morphogenetic execution are two different steps. NCCs may reach the correct region, but they still need a competent endodermal niche to expand and differentiate properly into cartilage. In this way, the pharyngeal endoderm acts as a local organizer. It turns tissue position with lineage-specific developmental outcomes[54](Fig. 2c).

3.1.4 Biomechanical and musculoskeletal inputs shape NCC derivatives

Mechanical regulation adds another important layer to these external controls, especially during skeletal morphogenesis. Anthwal et al. show this point through the mandibular coronoid process. Their comparative and genetic analyses suggest that the coronoid starts to form through a program within the ossifying bone, and this step requires factors such as Pax9. In contrast, its later growth depends on signals from the temporalis muscle attachment. Sox9 also supports this muscle-responsive growth, but it does so independently of chondrogenesis. This study is important because it shows that a craniofacial structure may begin through a neural crest-derived skeletal program, while its final shape can still be shaped by nearby musculoskeletal interactions. In other words, NCC derivatives are not fixed once they are specified. They continue to respond to biomechanical cues and tissue-contact cues during morphogenesis[55]. Antiguas et al. provide additional support for the importance of force and adhesion in craniofacial morphogenesis by showing cytoskeletal remodeling in both

ectoderm-derived epithelial cells and neural-crest-derived mesenchymal cells. During palatal shelf elevation, mesenchymal cells align their actin cytoskeleton, indicating actin-dependent contractility. At the same time, actomyosin dynamics also contribute to palatal shelf fusion through epithelial intercalation and extrusion[56]. Although this article is framed around orofacial clefts and adhesive biology, it has broader implications for neural crest regulation because it links tissue-level morphogenesis to mechanosensitive pathways and adhesive transitions. The activation of Rho GTPases and YAP during NCC EMT also points to a direct interface between mechanical context and cell-state change. Therefore, extrinsic control of cranial NCCs should be understood not only in terms of diffusible ligands such as WNT, BMP, FGF, TGFB1, or VEGF, but also in terms of tissue stiffness, contractility, ECM architecture, and muscle-generated load. These mechanical inputs may integrate with classical signaling pathways to determine whether NCCs migrate efficiently, survive, proliferate, or differentiate into stable craniofacial tissues[8] (Fig. 2d).

Overall, the literature supports a model of craniofacial development in which NCCs act as a central but deeply context-dependent lineage embedded within a multicellular regulatory network[57]. Signals from the ectoderm, mesoderm-derived endothelium, pharyngeal endoderm, and craniofacial musculature collectively orchestrate NCC induction, migration, survival, proliferation, and terminal differentiation[58]. Some of these external influences operate as morphogen gradients, others as ECM-mediated or hypoxia-related pathways, and still others as biomechanical cues transmitted through adhesion and muscle attachment. What unites them is that each modifies the developmental competence of NCCs at specific times and places, therefore ensuring coordinated assembly of the craniofacial complex[59]. A useful synthesis, therefore, is that cranial NCCs should be viewed not as isolated progenitors of facial structures, but as a responsive developmental interface through which diverse neighboring tissues come together to build the head[60]. This perspective has important implications for understanding craniofacial malformations, because disruptions in ectodermal, endothelial, endodermal, or musculoskeletal signaling may each secondarily distort neural crest behavior and produce similar anatomical outcomes through different developmental routes[61].

3.1.5 Ectoderm-neural crest interactions drive tooth morphogenesis

The craniofacial ectoderm does not develop in isolation; rather, organ morphogenesis is accomplished through its close association and reciprocal interactions with neural crest-derived mesenchyme, nerves, vasculature, and muscular tissues.

During tooth development, the oral ectoderm does not differentiate into a tooth independently; rather, its fate is step by step specified through continuous interactions with the surrounding embryonic environment, especially the cranial neural crest-derived ectomesenchyme[62]. In humans, migrating NCCs populate the first pharyngeal arch and establish the ectomesenchymal compartment beneath the primitive oral epithelium, thereby creating the tissue context required for odontogenesis[63]. The first visible sign of this process is the formation of the primary epithelial band and its invagination as the dental lamina. This occurs after the oral epithelium is positioned over neural crest-derived mesenchyme. At this early stage, the epithelium serves as the initial instructive tissue. It releases morphogens and growth factors, including SHH, FGFs, and BMPs. These signals induce condensation of the nearby ectomesenchyme and help establish the odontogenic field[64].

As tooth development moves from the bud stage to the cap stage, the epithelium no longer acts as the only leading tissue. Instead, the epithelium and mesenchyme begin to regulate each other through reciprocal signaling. Neural crest-derived ectomesenchymal cells condense around and beneath the epithelial bud. These cells form the dental papilla and dental follicle. These two structures do more than provide physical support; they also actively regulate epithelial morphogenesis[65]. During the cap stage, the enamel organ becomes more complex in structure. At the same time, the condensed mesenchyme receives and integrates signals from the primary enamel knot and inner enamel epithelium. The mesenchyme also begins to express key odontogenic transcription factors, including *Pax9* and *Msx1*. These factors are essential for sustaining further tooth morphogenesis[66]. This neural crest-derived input is important, as shown by the loss of mesenchymal *Msx1*. When mesenchymal *Msx1* is lost, tooth development stops around the bud or cap stage[67]. This change is also associated with reduced epithelial Bmp4 and Fgf3 signaling[68]. Thus, neural crest-derived mesenchyme is not a passive responder, but a decisive regulatory partner that feeds back to maintain epithelial growth, patterning, and cusp morphogenesis[68]. By the bell stage, the inner enamel epithelium exerts inductive effects on the peripheral dental papilla, promoting odontoblast differentiation. At the same time, the papilla in turn becomes functionally required for subsequent ameloblast maturation and enamel secretion. Fragmentation of the basement membrane facilitates this close epithelial–mesenchymal crosstalk, enabling the transition from morphogenesis to hard-tissue formation[69](Fig. 2e).

Recent human multi-omics evidence has further refined this view of tooth morphogenesis. Zhang et al. integrated single-cell RNA sequencing, spatial transcriptomics, and secretome analysis to construct a spatial and temporal atlas of human fetal tooth development from the late bud to early bell stages[70]. This study identified heterogeneous dental epithelial subpopulations with distinct gene expression profiles and spatial distributions, and showed that dental epithelial cells maintain extensive communication with dental mesenchymal compartments during development. In particular, the enamel knot and inner enamel epithelium showed close interactions with the coronal dental papilla, supporting a spatially organized epithelial-mesenchymal communication network during tooth morphogenesis and early hard-tissue formation. Secretome analysis further indicated that dental epithelium-derived factors participate in regulating mesenchymal cell fate and differentiation. Among these factors, epithelial WNT5B was enriched in the dental epithelial secretome and promoted odontogenic differentiation of human dental mesenchymal cells, as reflected by increased expression of odontoblast differentiation markers such as ALP, DMP1, and DSPP. These findings provide direct human developmental evidence that tooth morphogenesis is coordinated by dynamic and reciprocal communication between the dental epithelium and neural crest-derived dental mesenchyme[71].

Therefore, tooth development should be understood as a dynamic sequence in which dental epithelium and cranial neural crest-derived ectomesenchyme reciprocally regulate each other, with epithelial instructive signals, mesenchymal feedback, and stage-specific paracrine communication jointly shaping epithelial competence, morphogenesis, and final differentiation[70].

3.1.6 Neural and mesoderm-derived niches coordinate salivary gland epithelial morphogenesis

In the salivary gland, epithelial morphogenesis and lineage progression are also coordinated by extrinsic inputs that act at distinct developmental stages. A descriptive study of human embryonic submandibular gland development showed that the gland begins as an epithelial thickening in the floor of the mouth, then invaginates into condensed mesenchyme while the parasympathetic ganglion primordium and nearby vasculature become closely apposed to the developing epithelium, supporting the idea that gland development depends on coordinated interactions among these compartments. Although this study is primarily anatomical, it provides an important developmental framework in which the craniofacial epithelium is positioned as a target of extrinsic regulation rather than as an isolated self-patterning tissue[72].

Functional studies further show that parasympathetic nerves play instructive roles in shaping salivary gland epithelium. Nedvetsky et al. demonstrated that parasympathetic innervation regulates tubulogenesis in the embryonic salivary gland by promoting ductal growth, lumen continuity, and lumen expansion through VIP-dependent cAMP/PKA signaling; notably, this process involves CFTR-mediated fluid regulation rather than apoptosis-driven cavitation. Thus, neural input is not limited to later gland function but actively coordinates epithelial morphogenesis during organogenesis[73]. A second study moved this idea from duct morphogenesis to epithelial fate specification. Emmerson et al. showed that SOX2 is needed for acinar cell development, cell survival, and the expression of acinar-specific genes. They also showed that parasympathetic nerves regulate acinar formation by controlling SOX2[74]. These findings suggest that parasympathetic nerves affect both the structural assembly of the epithelial network and the differentiation of the secretory lineage. For this reason, parasympathetic nerves act as key regulators of salivary gland epithelial development.

The vasculature also exerts a clear instructive effect on salivary gland epithelial patterning. Using embryonic submandibular gland explants and reconstitution assays, Kwon et al. found that endothelial cells are required to maintain proper epithelial organization: depletion of CD31-positive endothelial cells, or inhibition of VEGFR2 signaling, increased cells expressing ductal markers K19 and K7 while reducing Kit-positive progenitor cells in endbuds. Importantly, epithelial patterning could be rescued either by reintroducing endothelial cells or by supplementing endothelial cell-regulated mesenchymal factors such as IGFBP2 and IGFBP3, indicating that vascular regulation is mediated through signaling rather than perfusion alone. These data suggest that, in the salivary gland, epithelial morphogenesis and lineage balance are jointly constrained by neural and endothelial niches[75] (Fig. 2f).

3.1.7 Epithelial-intrinsic programs and neural inputs coordinate taste bud development

The situation is more detailed for taste bud development. Several classical studies indicate that early taste bud differentiation can occur without nerve contact. In amphibian experiments, Barlow et al. showed that embryonic taste buds can differentiate fully in grafts and explants lacking normal innervation, indicating that innervation is not required for initial taste receptor cell differentiation[76]. Similarly, Barlow and Northcutt found that taste buds can develop in tissue lacking cephalic neural crest and paraxial mesoderm derivatives, arguing against an essential inductive role for those surrounding tissues in early embryonic taste bud formation[77]. In mammals, Thirumangalathu et al. further showed that *Shh*-expressing embryonic taste placodes are progenitors of taste bud cells. In contrast, neural crest does not contribute cells to taste buds, and early progenitor specification and differentiation can still occur in the absence of gustatory nerve contact[78]. Taken together, these studies support the conclusion that early taste bud progenitor specification is largely epithelial-intrinsic and does not require direct neural crest contribution.

However, more recent mammalian evidence indicates that nerves are nonetheless crucial for proper taste bud organogenesis. Fan et al. genetically ablated oral sensory neuron populations in mice and showed that visceral gustatory neurons, but not somatic sensory neurons, are required for proper taste bud formation. This suggests that although early epithelial specification may begin independently, later stages of mammalian taste bud formation are nerve-dependent[79]. In parallel, epithelial signaling itself is indispensable: Liu et al. demonstrated that WNT/β-catenin signaling in lingual epithelium is required to initiate fungiform papilla morphogenesis, with β-catenin activation causing excess papillae and taste buds, and β-catenin loss or *Dkk1* expression blocking papilla formation[80]. Overall, the studies support a comparative conclusion: salivary gland epithelium is directly regulated by parasympathetic nerves and endothelial cells during morphogenesis and lineage formation, whereas taste bud development relies strongly on intrinsic epithelial patterning at early stages but, at least in mammals, later becomes dependent on gustatory innervation (Fig. 2g).

3.1.8 Neural crest–mesoderm interactions in patterning craniofacial musculature

During craniofacial development, mesoderm-derived muscle formation is shaped by a series of tissue–tissue interactions rather than by an independent myogenic program[2]. In the head, cranial mesoderm develops in an environment that is structurally and molecularly distinct from the trunk: it is surrounded by neural and surface epithelia, pharyngeal endoderm, and—most importantly for the studies discussed here—cranial neural crest-derived mesenchyme. Noden and Francis-West emphasized that craniofacial muscle morphogenesis takes place within this unusually complex tissue context, and that the progressive association of neural crest-derived connective tissues with myogenic populations coincides with myocyte alignment, muscle segregation, and later musculoskeletal integration[12].

These studies lead to a central conclusion. CNCCs are not just passive neighbors of mesoderm-derived muscle precursors. They also actively regulate how these precursors are patterned and how they differentiate. Rinon et al. showed that the earliest steps of head myogenesis can occur without cranial neural crest input. Later steps, however, depend on neural crest-derived regulation. These steps include migration, spatial patterning, and terminal differentiation of muscle precursors. When neural crest input was absent, myoblasts accumulated but stayed in a proliferative state. This defect was linked to increased Fgf8 in nearby tissues. As these cells failed to leave the progenitor state, myofiber organization was ultimately disrupted[81].

This regulatory role is closely linked to the unusually intimate physical relationship between CNCCs and cranial mesoderm.

Grenier et al. revised the earlier view that CNCCs merely surround the mesodermal core of the branchial arches, showing instead that CNCCs enter the cephalic mesoderm containing myogenic progenitors in the first branchial arch[82]. The same study demonstrated that the tendons associated with branchiomeric and extraocular muscles are neural crest-derived and express Scleraxis, reinforcing the idea that neural crest establishes much of the connective tissue context in which muscle develops. Moreover, although tendon initiation could occur in the absence of branchiomeric muscles, later tendon development required the presence of muscle, suggesting a reciprocal relationship between these two lineages[83]. These results imply that cranial mesodermal muscle development is regulated not only by soluble signals but also by its incorporation into a neural crest-derived scaffold of connective tissues and attachment structures. In this way, the neural crest contributes both molecular signals and architectural context, helping transform mesodermal precursor populations into regionally organized and functionally connected craniofacial muscles[84].

The tongue offers one of the clearest examples of how this tissue interaction operates at the molecular level. Hosokawa et al. showed that CNCCs give rise to fibroblastic components of tongue skeletal muscle and that loss of *Tgfbr2* in these CNCCs causes microglossia, reduced *Scx* and *Fgf10* expression, decreased proliferation of myogenic cells, and disorganized tongue musculature. Crucially, *Fgf10* supplementation rescued the muscle cell number defect, indicating that TGF-β-dependent signaling in neural crest derivatives supports mesoderm-derived myogenic progenitors through FGF-mediated crosstalk[85]. In addition, Iwata et al. found that disruption of TGFβ signaling in CNCCs impairs the differentiation of neural crest-derived fibroblastic cells and secondarily reduces the proliferation and differentiation of mesoderm-derived myoblasts during tongue development[86]. These findings show that CNCCs control cranial mesodermal myogenesis not only by being present in the local microenvironment, but by acquiring specific fibroblastic identities that produce signals essential for myoblast expansion and differentiation. In other words, the cranial neural crest acts as a signaling intermediary that translates tissue context into specific developmental instructions for mesoderm-derived muscle.

This concept is reinforced by Han et al., who examined ALK5-mediated TGF-β signaling in CNCCs and showed that defects in this pathway severely disrupt craniofacial muscle formation through altered tissue–tissue interactions. In their model, increased Bmp4 was associated with reduced proliferation of myogenic progenitors, whereas reduced Fgf4 and Fgf6 impaired myogenic differentiation. Rescue experiments confirmed that BMP and FGF pathway manipulation could partly restore these defects, supporting a model in which CNCCs regulate both the proliferative and differentiation phases of craniofacial myogenesis[87] (Fig. 2h).

We added a summary table to synthesize the participating lineages, representative mechanisms, and developmental examples associated with each coordination mode (Table 1).

3.2 Integrated signaling pathways in multilineage craniofacial coordination

The tissue-level examples discussed above show that craniofacial morphogenesis depends on repeated signaling modules, not only on which lineage contributes each tissue. TGFβ1-dependent ECM remodeling links mesoderm-derived endothelium with NCC survival, migration, and osteogenic differentiation[52–53]. The Klf5a–FGF axis connects pharyngeal endoderm with NCC-derived cartilage formation[54]. SHH, BMP and FGF signals support epithelial–mesenchymal crosstalk during tooth morphogenesis[64,68]. Epithelial WNT/β-catenin signaling starts taste papilla development[80]. FGF, BMP and TGFβ signals from neural crest-derived connective tissues also help pattern mesoderm-derived craniofacial muscles[85-87].

These observations suggest that Hedgehog, WNT, BMP, FGF, and TGFβ-related pathways should not be viewed as separate pathway lists. These pathways act as context-dependent regulators during craniofacial construction. They connect epithelial, mesenchymal, endothelial, endodermal, neural, and myogenic compartments in different developmental settings[52–53,64,80,85-87].

Among these pathways, BMP signaling provides a clear example of integrated control. Its developmental effect depends on dosage,

Table 1 Major modes of multilineage coordination during craniofacial morphogenesis.

Coordination mode	Main participating lineages	Representative mechanisms	Developmental examples
Epithelial–mesenchymal interaction	Surface ectoderm, pharyngeal endoderm, NCC-derived mesenchyme	Epithelial signals regulate NCC induction[11,44,46,88], migration, proliferation and differentiation[51,54]	NCC EMT, tooth morphogenesis[44,46,8,88], palate fusion[51], pharyngeal cartilage[54]
Biochemical signaling	Ectoderm, endoderm, NCCs, endothelium, mesoderm	WNT[8,51,70,80], BMP[12,64,87], FGF[12,54,64,81,85,87], SHH[68], TGFβ[52,85-87], VEGF[57] send positional and fate-specifying information	Neural crest induction[8,12,88], tooth development[64-66,68], , muscle patterning[2,81–82,84]
Biomechanical/ ECM regulation	NCCs, epithelium, endothelium, muscle	Adhesion remodeling[8], cytoskeletal dynamics[8], ECM stiffness[8,46], Rho/YAP, muscle-generated force[55]	NCC migration[8,46,52], palatal shelf elevation/fusion[8], coronoid process growth[55]
Neurovascular niche interaction	Endothelium, nerves, epithelial progenitors, NCC-derived mesenchyme	TGFβ1–ECM remodeling[52], VEGF/HIF1α signaling[53], VIP–cAMP/PKA[73], SOX2 regulation[74]	NCC survival/migration[52], craniofacial ossification[53], salivary gland morphogenesis[72-75], taste bud maturation[76-80]
Musculoskeletal coupling	NCC-derived connective tissue, cranial mesoderm-derived muscle	TGFβ–FGF/BMP crosstalk[85-87], tendon–muscle interaction[82,84], mechanical loading[55]	Tongue muscle development[85-87], branchiomeric muscle patterning[2,81–82,84], skeletal–muscle integration[82,84]

Abbreviations: NCC, neural crest cell; EMT, epithelial-to-mesenchymal transition; ECM, extracellular matrix; WNT, Wingless-related integration site; BMP, bone morphogenetic protein; FGF, fibroblast growth factor; SHH, Sonic hedgehog; TGFβ, transforming growth factor beta; VEGF, vascular endothelial growth factor; HIF1α, hypoxia-inducible factor 1 alpha; Rho, Ras homolog family member GTPase; YAP, Yes-associated protein; VIP, vasoactive intestinal peptide; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; SOX2, SRY-box transcription factor 2.

timing, receptor context, and cellular compartment. In cranial NCCs and suture-associated mesenchyme, abnormal BMP activity can change proliferation, apoptosis, differentiation, and fate specification. In this way, BMP signaling links morphogen regulation with orofacial clefting and craniosynostosis[88].

At the cranial suture, WNT/β-catenin, FGF and BMP signaling work together to regulate the fate of mesenchymal stem/progenitor cells. These signals influence whether the cells undergo osteogenic differentiation or move toward chondrogenesis and endochondral ossification. Studies have shown that changes in WNT/β-catenin and FGF signaling can promote ectopic chondrogenesis. This finding shows that signaling crosstalk is important for normal suture development and skeletal lineage specification[89].

Studies of Axin2-positive suture stem cells further support this concept. These studies show that BMPR1A-dependent BMP signaling is needed to maintain the suture stem-cell niche and normal suture homeostasis. The loss of Bmpr1a in these cells causes stem-cell depletion and craniosynostosis. This finding shows that BMP signaling has a context-dependent role in balancing stem-cell maintenance and osteogenic differentiation[90].

The WNT/β-catenin pathway also needs a more careful interpretation. Its role in skeletal lineage specification is not limited to canonical LEF/TCF-dependent transcription. Maruyama et al. showed that β-catenin can regulate craniofacial skeletal fate through a nonclassical mechanism involving GATA3. This finding suggests that different β-catenin outputs help guide osteogenic and chondrogenic lineage decisions[91].

A second level of pathway integration occurs intracellularly, where enhanced BMP signaling can increase WNT/β-catenin activity through mTORC1-dependent suppression of autophagy in CNCC-derived craniofacial tissues. This BMP-mTORC1-autophagy-WNT/β-catenin axis links BMP receptor activity to β-catenin regulation and influences CNCC fate decisions, including aberrant chondrogenic differentiation and ectopic craniofacial cartilage formation[92].

BMP signaling also connects CNCC behavior with metabolic and epigenetic regulation during midfacial morphogenesis. Yang et al. showed that excessive BMP/Smad signaling in CNCCs disrupts a p53-dependent metabolic pathway. This change reduces lactate production and histone lactylation, and it leads to severe midline facial defects. These changes impair PDGFRα-associated CNCC growth and migration. This finding suggests that BMP signaling can affect craniofacial morphogenesis through metabolic and epigenetic mechanisms that change how CNCCs respond to growth-factor signals[93].

TGFβ-related regulation can also be included in this integrated view. This regulation interacts with inhibitory SMADs and other signaling pathways that help maintain suture homeostasis. Ueharu and Mishina showed that inhibitory SMADs regulate TGFβ-superfamily signaling and help control cranial suture patency. These findings suggest that BMP, FGF, and TGFβ-related pathways work through shared regulatory networks during suture morphogenesis[88].

Taken together, craniofacial morphogenesis is governed by pathway balance rather than pathway presence alone. We added a summary table to provide a clearer overview of the major integrated signaling axes and their roles in multilineage craniofacial coordination (Table 2).

To provide a clearer organ-level overview of multilineage coordination, we added a summary table comparing the major lineage contributions, representative coordinating interactions, developmental outcomes, and disease relevance across craniofacial organs and structures (Table 3).

4 Conclusion and perspective

Craniofacial development is now best understood as a systems-level process that emerges from the coordinated interactions of CNCCs, surface ectoderm, mesoderm, and pharyngeal endoderm, rather than from the autonomous behavior of any single lineage[94]. As highlighted throughout this review, neural crest-derived mesenchyme provides much of the craniofacial skeletal and

Table 2 Major signaling pathways integrated in multilineage craniofacial coordination.

Integrated pathway/axis	Main developmental context	Coordinated lineages or compartments	Key integrative role
SHH–BMP–FGF epithelial–mesenchymal signaling[70,77]	Tooth morphogenesis and craniofacial ectodermal organ development[69–70,77]	Surface/oral epithelium and NCC-derived ectomesenchyme	Coordinates epithelial patterning with mesenchymal responses during organ initiation, morphogenesis, and differentiation[70,77].
WNT–FGF–BMP balance[89]	Cranial suture morphogenesis[89]	Suture mesenchymal stem/progenitor cells and osteogenic fronts	Regulates skeletal precursor expansion and determines whether suture mesenchyme follows osteogenic or chondrogenic/endocondral programs[89].
BMP–BMPR1A suture stem-cell signaling[90]	Calvarial suture niche[90]	Axin2+ suture stem cells and osteogenic derivatives[90]	Maintains SuSC stemness, self-renewal, clonal expansion, and SuSC-mediated bone formation, thereby preserving suture homeostasis[90].
BMP–mTOR/autophagy–WNT/β-catenin and β-catenin–GATA3 signaling[91–92]	CNCC-derived craniofacial skeletal fate specification[91–92]	CNCC-derived craniofacial mesenchyme and cartilage-forming progenitors[91–92]	Links BMP signaling to β-catenin stability and nonclassical β-catenin output, thereby controlling osteogenic versus chondrogenic fate decisions[91–92].
BMP–p53–lactate/histone lactylation–PDGFRα and TGFβ-related signaling[59,88,93]	Midfacial morphogenesis, endothelial–NCC interaction, and suture regulation[88,93]	CNCC-derived nasal process mesenchyme[93], mesoderm-derived endothelium, ECM[59], and suture cells[88]	Couples BMP activity to CNCC metabolism, epigenetic regulation, PDGFRα-dependent growth/migration[93], and TGFβ-related ECM[59]/suture regulatory mechanisms[88].

Abbreviations: BMP, bone morphogenetic protein; BMPR1A, bone morphogenetic protein receptor type 1A; CNCC, cranial neural crest cell; ECM, extracellular matrix; FGF, fibroblast growth factor; GATA3, GATA binding protein 3; mTOR, mechanistic target of rapamycin; NCC, neural crest cell; PDGFRα, platelet-derived growth factor receptor alpha; SHH, Sonic hedgehog; SuSC, suture stem cell; TGFβ, transforming growth factor beta; WNT, Wingless-related integration site.

Table 3 Summary of lineage contributions and disease relevance across craniofacial organs and structures.

Organ/structure	Major contributing lineages	Main coordinated interaction	Developmental outcome	Disease/translational relevance
Craniofacial skeleton	CNCCs; mesoderm-derived endothelium and muscle; surface ectoderm	NCC–endothelial signaling[52–53]; biomechanical input[8,55]; epithelial patterning cues[51]	Intramembranous ossification and craniofacial bone patterning[51,53,55]	Craniofacial dysmorphology[51], micrognathia[53], skeletal malformations[88,91-93]
Pharyngeal cartilage/arches	CNCCs; pharyngeal endoderm; mesoderm; ectoderm	Endodermal niche signaling, including FGF-related regulation[54]	NCC proliferation, chondrogenesis, and arch patterning[30]	Pharyngeal arch defects, jaw and hyoid cartilage abnormalities[22,30,54]
Palate	CNCC-derived palatal mesenchyme; ectoderm-derived palatal epithelium; mesodermal and vascular support	Epithelial–mesenchymal crosstalk[23]; epithelial seam formation and breakdown[23]; biomechanical regulation[8]	Palatal shelf growth, elevation[23], fusion, and mesenchymal continuity[8]	Cleft palate[8,23,53] and orofacial clefting[8,51]
Tooth	Oral ectoderm; CNCC-derived ectomesenchyme; nerves and vasculature	Reciprocal epithelial–mesenchymal signaling[64-66,68-70], including SHH[32,64], BMP, FGF[64,68], WNT-related interactions[32,70]	Dental lamina formation[63–64], tooth germ morphogenesis[64-66,68–69], odontoblast/ameloblast differentiation[69–70]	Tooth agenesis, enamel/dentin defects[66,69,70]
Salivary gland	Oral epithelium; mesenchyme; parasympathetic nerves; endothelial cells	Neural and vascular niche regulation[73], VIP, VEGF, and IGFBP-mediated signaling[75]	Branching morphogenesis, duct formation, lumen expansion[73], acinar differentiation[74]	Salivary gland hypoplasia, xerostomia[72-75]
Taste bud	Oral epithelium; gustatory nerves; limited or no direct CNCC contribution to taste receptor cells	Early epithelial-intrinsic specification followed by nerve-dependent maturation and maintenance[76-79]	Taste placode specification and taste bud maturation[76-80]	Taste dysfunction, sensory epithelial maintenance and regeneration[76-80]
Craniofacial muscle	Cranial mesoderm; CNCC-derived connective tissues; nerves and vasculature	NCC–mesoderm interaction; connective-tissue-mediated myogenic patterning[2,12,81–82,84-87]	Muscle alignment, attachment, and functional musculoskeletal integration[2,12,81–82,84-87]	Muscle patterning defects, craniofacial movement disorders[81,85-87]
Cranial suture/calvaria	CNCCs and mesoderm-derived skeletal progenitors; dura mater; suture mesenchyme	Osteogenic signaling balance involving BMP, FGF, WNT, TGFβ-related regulation[88-92]	Suture patency, calvarial bone growth, and osteogenic differentiation[88-92]	Craniosynostosis and calvarial regeneration[88-92]

Abbreviations: BMP, bone morphogenetic protein; CNCC, cranial neural crest cell; FGF, fibroblast growth factor; IGFBP, insulin-like growth factor-binding protein; NCC, neural crest cell; SHH, Sonic hedgehog; TGFβ, transforming growth factor beta; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide; WNT, Wingless-related integration site.

connective framework, but its developmental potential is continuously shaped by epithelial signaling, endothelial and mesodermal support, endodermal niche cues, and biomechanical regulation[3]. This perspective helps explain why disruptions arising in different tissues can converge on similar craniofacial malformations: the phenotype often reflects failure of inter-lineage coordination rather than a defect confined to one cell type[95].

A major challenge for the field now is to move from descriptive maps of cell types and signaling pathways to causal and predictive models of tissue coordination. Recent single-cell and spatial datasets have begun to resolve previously hidden heterogeneity within craniofacial mesenchyme, ectoderm, and epithelial subdomains, and have shown that anatomically discrete cell populations contribute differentially to normal facial patterning and to the risk architecture of disorders such as orofacial clefting[94]. At the same time, transcriptomic and epigenomic studies have identified more candidate craniofacial disease genes and developmental enhancers. These studies also show that disease-related changes may occur not only in coding sequences, but also in gene regulatory regions. These advances suggest that researchers need to connect lineage-resolved cell states, enhancer activity, and intercellular signaling into developmental pathways that can be tested in experiments. This work will help explain how short-term embryonic interactions produce stable morphological outcomes. It will also help explain why similar malformations can result from defects in ectodermal signaling, endothelial patterning, endodermal organization, or neural crest competence[96].

This point also has value for clinical translation. Developmental principles derived from multilineage coordination suggest that craniofacial diseases should increasingly be viewed as disorders of tissue interaction, niche dysfunction, or regulatory imbalance. This has practical significance for congenital anomalies, because identifying the affected lineage alone may not be sufficient unless the disrupted epithelial, vascular, neural, or matrix context is also defined[97].

These concepts also offer important guidance for regenerative medicine. Reconstructing craniofacial tissues will require more than replacing cells or filling defects with biomaterials; it will require rebuilding the developmental interactions that normally coordinate skeletal, epithelial, vascular, neural, and glandular assembly[98]. In this regard, organoid, assembloid, and stem cell-based platforms provide promising avenues for modeling human craniofacial development, testing disease mechanisms, and designing more faithful regenerative strategies[99].

Overall, the most important implication of this field is that craniofacial construction is fundamentally an emergent property of coordinated multicellular development[100]. A deeper mechanistic understanding of this coordination will not only refine developmental theory, but also provide a stronger foundation for precision diagnosis, disease modeling, and next-generation therapies for craniofacial defects and tissue loss.

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