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HUMAN LIMB DEVELOPMENT ON THE 3 AXES OF COORDINATES. MOLECULAR ETIOLOGY OF POLYDACTYLY

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Abstract

Limb development is a very complex process, not completely understood yet. There are multiple genes implicated during the process and the complexity of limb development consists in the interaction of these genes through transcription factors. The responsible structures for limb development are the centres of signalling, areas of the limb bud where gene transcription has a high rate and transcription factors determine the pattern of the cells situated nearby. The Apical Ectodermal Ridge (AER) is the signalling centre responsible for the proximal-distal development of the limb; the Zone of Polarizing Activity (ZPA) is responsible for the patterning of the limb on the anterior-posterior axis. The non –AER ectoderm is responsible for the dorsal-ventral pattering.

Key words: Limb development, centres of signalling, transcription factors, apical ectodermal ridge, zone of polarising activity

INTRODUCTION

The most important processes of limb development take place in the fifth to eight week of intrauterine life at the level of limb buds.

During embryological development the upper and lower limb present 3 segments: the proximal segment named stylopode, made of a single skeletal element (hummers/femur), the middle segment named zygopode, made of two skeletal elements (radius-ulna/tibia-fibula), the distal segment the autopode containing the numerous skeletal elements of the hand and foot (Crivii Carmen, 2008).

Upper limbs and the lower limbs go through the same stages of morphogenesis, with the observations that the upper limb develops faster than the lower limb with 1 or 2 days (Mankin, 2011).

Understanding the processes that take place during limb development would be impossible without knowing the role of transcription factors and of paracrine factors. In a way we can tell that genes communicate through transcription factors and paracrine factors.

MATERIAL AND METHODS

We reviewed data from the literature and identified the main centres responsible for limb development. We also explained the complex interaction between the centres of development that ensure a progressive and coordinated growth of the limb.

RESULTS AND DISCUSSION

Diffusive proteins responsible for paracrine signalling are called paracrine factors or factors of growth and differentiation (FGD).

Signal transduction is realized by a signal molecule named ligand and a receptor that crosses the cellular membrane having an extracellular domain where the ligand attaches, a transmembrane domain and a cytoplasmic domain.

The ligand determines the conformational modification of the structure of the receptor by attaching to it, which will determine the activation of the cytoplasmic domain. The receptor will have enzymatic activity as a consequence of this activation, usually protein-kinase activity, having the capacity to phosphorylate another proteins, in a way in which it will form a cascade of interactions that will lead to activation of a transcription factor. Transcription factors activate or inhibit the transcriptions of certain genes (Sandler, 1995).

Limb buds are formed of mesoderm and ectoderm: a mesenchymal portion at the inside, derivate form the somatopleural mesodermal plate, and an ectodermal covering at the outside.

Under the inductor influence of the underlying mesenchyme, more specifically of the Fibroblastic Growth Factors, in the ectoderm that covers the distal portion of the limb bud, starts to differentiate an epithelial thickening called the Apical Ectodermal Ridge (AER). This ridge will influence the underlying mesenchyme, so that the mesenchyme next to the ridge will remain undifferentiated while cells situated far from the AER will start to differentiate in cartilaginous, osseous or muscular structures (Schwabe, 2004).

Limb development model is under the control of a group of cells situated in the posterior region at the base of the limb – the Zone of Polarizing Activity- that creates a morphogenetic gradient. Zone of Polarizing Activity and Apical Ectodermal Ridge are the two main centres of organizing of the limbs that regulate cell proliferation and growth according to the axis of development which they control. Their activity is regulated through positive feedback.

Cellular and molecular bases of limb development

Limb development is realized on three main axes.
- proximal - distal axis
- anterior - posterior axis
- dorsal - ventral axis

Genes are responsible for:
- determining the exact position where the limb will form
- determining the identity of the limb
- determining the pattering on the anterior-posterior axis (from the hallux to the small finger)
- determining the patterning on the proximal-distal axis (from the shoulder to the phalanx)
- determining the patterning on the dorsal-ventral axis (from the dorsal to the palmar face of the hand)

Only a small portion of the lateral mesodermal plate has the ability to generate limbs. There must exist a constant equilibrium between mitotic and apoptotic activity. The ability to start the development of the limb is gained by the lateral mesoderm because of the activity of HOX genes and axial positioning indices (Biesecker, 2011).

HOX genes are expressed on the anterior-posterior axis of all segmented animals and are fundamental for limb positioning. Limb buds responsible for limb development will form from the lateral mesoderm. The process is mediated by the activation of HOX genes. HOX genes are activated by retinoic acid. After the signalling starts, meaning after the mesenchyme can generate limb, it will start the synchronous and progressive growth on the 3 axes of coordinates: anterior-posterior, dorsal-ventral and proximal-distal. 3 genes were identified that are responsible for limb identity. TBX5 and TBX4 are transcription factors that belong to the T-BOX family. These genes that encode for TBX5 and TBX4 are expressed at the level of upper limb buds and lower limb buds. PITX1 is a gene that is expressed only at the level of the lower limb. Genetic patterns of temporal and spatial expression of TBX4, TBX5 and PITX1 suggest that they play an important role in programming the identity of the limb during the process of development (Stoll, 2001).

Apical Ectodermal Ridge and control of development on proximal-distal axis

The Apical Ectodermal Ridge is the main centre of signalling for the development of the limb bud. Experiments done on chicken embryos showed that if the AER is removed the limb will be truncated transversally.

The degree of truncation will depend of the embryological age when the AER is removed (Schwabe, 2004).

The Apical ectodermal Ridge has the next functions:

- The secretion of FGF8. To maintain the mesenchyme in an active mitotic state. Through secretion of FGF8 the AER sends a message to the mesodermal cells to continue proliferation assuring this way the development on the proximal-distal axis.
- To regulate the activity of the other signalling centres FGFs secreted by the Apical Ectodermal Ridge influence the Zone of Polarizing Activity. FGF maintains the secretion of Sonic Hedgehog by the Zone of Polarizing Activity. Zone of Polarizing Activity is implicated along with the HOX genes in the patterning of the limb on the proximal-distal axis. SHH activates GREMLIN, which produces a transcription factor that inhibits BMP. BMP are transcription factors that stop limb development by their inhibitory activity on FGF at the level of the Apical Ectodermal Ridge. This way the Zone of Polarizing Activity and the Apical Ectodermal Ridge sustain each other through a loop of positive feed-back that implies FGF, SHH, GREMLIN4.

Anterior-posterior axis: Zone of Polarizing Activity and Sonic Hedgehog signalling pathway

Anterior-posterior polarity of the limb bud depends of Zone of Polarizing Activity, this being localised in the post-axial region of the limb bud. It was demonstrated that Sonic Hedgehog is the protein responsible for the anterior-posterior polarity and for specifying the digital pattern, meaning in case of humans the shape of every digit from I to V. Hedgehog gene produces a diffusive protein that acts as a morphogen and is implicated in intercellular signalling (Talamillo, 2005).

Another gene GLI3 is important for fingers development. GLI3 has an activating form (GLI3A) implicated in the SHH signal transduction and a repressor form (GLI3R). Balance between GLI3A and GLI3R decides digit number and identity. In the presence of SHH processing is stopped, which leads to an increased level of GLI3R anteriorly and a decreased level of GLI3A posteriorly (Malik, 2012).

SHH has the role to inhibit GLI3 repressor activity. SHH migrates from the level of Zone of Polarizing Activity, having an increased level in the posterior region of the limb bud. GLI3A will predominate in the posterior region of the limb bud while GLI3R is active in the anterior region. GLI3A activates transcription of genes like HOX, FGF and BMP in the posterior region of the limb bud, starting digit patterning. Especially

HOXA and HOXD are probably controlled by SHH at the level of Zone of Polarizing Activity. HOX genes are expressed in a grouped manner and are responsible for digit shape and identity.

Dorsal-ventral patterning

Many transcription factors are believed to be implied in the signalling pathways that determine the development on the dorsal-ventral axis of the limb in humans, as in the others development axes. Many of them are still unknown. If the gene that codes transcription factor Engrailed (EN1) is mutated a double dorsal phenotype appears, and if the gene that codes transcription factor Wingless-related MMTV integration site7A (WNT7A) is mutated or LIM homeobox transcription factor-1 (LMX1B) is mutated a double ventral phenotype appears.

The dorsal ectoderm of the limb bud produces WNT7A which is repressed in the ventral ectoderm by EN1.

Cartilaginous and osseous development progression and the role of morphogenetic proteins

HOX genes are responsible for osseous morphogenesis at the level of the limbs during the process of limb development. SHH, FGF and WNT all promote HOX gene expression. HOXA and HOXD are the most important genes for limb development.

BMP (Bone morphogenetic proteins) are responsible for stopping the activity of FGF so they stop the activity on the proximal-distal axis. BMP activity is inhibited by Sonic Hedgehog signalling pathway. BMP play an essential role in morphogenesis, cartilaginous differentiation and apoptosis in the interdigital spaces. BMP2, BMP4 and BMP7 are essential for mesodermal differentiation (Mankin, 2011).

Congenital digital anomalies represent somatic genetic markers because their presence shows the existence of genetic mutations, and genes that control limb development and in consequence digit development, play an important role in embryogenesis at the level of the whole body. Currently there are known 84 genes implied in the apparition of congenital digital anomalies. Congenital malformations that accompany congenital digital anomalies, some of them very severe, justify the knowing of genes implied in their apparition. So the newborn with a congenital digital anomalies represent a challenge for the geneticist in the understanding of the process of the apparition of the anomaly, detecting additional anomalies, assessing the modality of transmission of the genetic mutation, analysis of the risk of recurrence and establishing the prognosis.

Tabel 1.

Explanations of the apparition of congenital anomalies

Digital anomaly	Possible genetic explication of apparition of congenital anomaly
Polydactyly	Responsible axis: anterior-posterior axis with the center of signaling: Zone of Polarizing Activity, mutation of GLI3, mutation of genes that are responsible for transcription factors formation implied on the Sonic Hedgehog pathway
Syndactyly	BMP signaling pathway (Bone morphogenetic protein) responsible for interdigital apoptosis
Olygodactilies, Bracydactylies	Responsible axis: posterior-distal axis, with center of signaling apical ectodermal ridge, genes that produce FGFs (fibroblast growth factors)

Polydactyly

SHH Signaling Pathway

Sonic Human Hedgehog (SHH) is a protein member of the Hedgehog family, a family of intercellular signaling proteins, and functions as a key morphogen in a dose dependent manner to induce the cellular type, to ensure the growth and cellular typing of different tissues. Sonic Hedgehog proteins play a role in many steps of the development, that include limb bud development, neural tube induction and development, somite differentiation, intestinal segmentation and others. The cell receptor for proteins in the Hedgehog family is named Patched, and this receptor is attached to a protein named Smoothened. Smoothened protein makes the transduction of hedgehog signaling, but is inhibited by Patched until the moment when a hedgehog protein is attached by this receptor. So, the role of the paracrine factor hedgehog is to attach itself by its specific receptor to stop the inhibition of a transductor molecule that otherwise would be active and not to activate directly this molecule (Philip-Sarles, 2008).

GLI1, GLI2, GLI3 are 3 proteins present in vertebrates represent the effectors of Sonic Hedgehog protein transcription. In the absence of SHH ligand, GLI2 and GLI3 proteins are found in a repressor form with reduced size, migrate at the level of the nucleus and block genetic transcription of target genes of SHH. In the presence of SHH ligand, normal size GLI proteins migrate at the level of the nucleus and functions as activator of genetic transcription to induce the genetic expression of target genes of SHH (Schwabe, 2004).

Mouse embryo analysis shows that GLI3 gene plays an important role in limb patterning. Mice with GLI1 and GLI2 mutations have normal limbs but the one with GLI3 mutations have limbs with polydactyly. In the normal limb, the repressor form of GLI3 gene (GLI3R) is present at a low level in the posterior region of the limb, where fingers will develop, and at the level of the anterior region GLI3R level is increased, here there will not develop fingers. Based on this data it was concluded that the main role of SHH signaling is to create a GLI3 gradient at the level of limb bud, maintaining low levels of GLI3R al the level of the posterior region and increased levels of GLI3R at the level of the anterior margin.

CONCLUSION

HOX genes give the lateral mesenchyme the capacity to generate limbs. FGF initiate the forming of the Apical Ectodermal Ridge, of Zone of Polarizing Activity and WNT7A initiate dorsal-ventral development. The Apical Ectodermal Ridge sends the growth signals in the underlying mesenchyme, and the mesenchyme ensures the stability and persistence of the Apical Ectodermal Ridge. This is the mechanism responsible for the growth in length of the limb. The Zone of Polarizing Activity determines the patterning of the limb from the anterior level to the posterior level trough the diffusible protein SHH, which maintains a low level of GLI3R at the posterior margin and a high level of GLI3R at the anterior margin of the limb bud. The activity of genes HOXA and HOXD is inhibited by the high level of GLI3R and their function manifests only in the presence of low GLI3R levels. BMP genes inhibit FGF and indirectly the activity of Zone of Polarizing Activity, only HOX and BMP genes will remain active in the late stages of limb development and these genes will be responsible for bone formation and cartilaginous differentiation, processes that will bring the limb bud closer and closer to its mature form.

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