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Notch signaling pathway and tissue engineering

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1. ABSTRACT

The Notch pathway is a signaling network essential for proper organ development in an embryo, and is indispensable for tissue regeneration in the adult. This key regulatory signaling network is evolutionarily conserved in all metazoans and is continually utilized for the building, maintenance and repair of diverse organs and tissues. Importantly, dysfunctions in the Notch pathway have been demonstrated to result in oncogenic transformation, such as in lymphoid cancers, and have been linked to the pathogenesis of several inherited human diseases. Therefore, the ability to regulate Notch signaling intensity both positively and negatively has a very high therapeutic relevance. Adapting this pathway for tissue engineering applications has great potential to spear-head the development of smart biomaterials to deliberately control cell-fate decisions and lead to designer *ex vivo* morphogenesis. This review describes the components of Notch-specific signal transduction, presents the role of the Notch signaling network in constructing and repairing multiple organ systems, summarizes the Notch-related pathologies, outlines current advances in the deliberate modulation of the Notch pathway in bioengineering applications, and introduces future perspectives on the use of Notch pathway manipulations as a powerful universal tool in tissue engineering and in the orchestration of stem cell responses. This review also summarizes the existing bioengineering methods most suitable for the deliberate manipulation of Notch signaling, such as smart biomaterials able to pattern Notch ligands or to create gradients of Notch agonists and antagonists. Such methods will likely facilitate the engineering and dynamic remodeling of tissues composed of stem, progenitor and differentiated cells derived from an initially equivalent cell population.

2. INTRODUCTION

Notch was discovered in *Drosophila melanogaster* in the early 20th century and received its name from the "notched" wing phenotype in flies bearing a mutation in this locus (1-3). Notch was one of the first genes proven to regulate early embryonic development, and the term "neurogenic" has been coined to describe one of the Notch mutations (4). Specifically, the activity of this pathway has been shown to control the balance between epidermal and neural cell-fate determination, where a lack of Notch activity results in an excess of neuroblasts and neural tissue and insufficient epidermal tissue in *Drosophila melanogaster* (4).

It was later established that the Notch pathway is extremely well conserved between flies and worms (*Caenorhabditis elegans),* where Notch/LIN-12 proteins regulate formation of organs in early embryonic development and, generally speaking, control cell fate determination (5). The regulatory role of Notch on cell fate determination and the molecular mechanisms by which it controls proliferation and differentiation of stem and progenitor cells in all three germ layers have also been extensively studied in vertebrates, including zebrafish, *Xenopus*, birds, mice and humans, and have been found to be well conserved (6-13).

3. THE NOTCH SIGNALING PATHWAY, ITS ROLE IN EMBRYONIC DEVELOPMENT AND POTENTIAL APPLICATIONS FOR *EX VIVO* MORPHOGENESIS AND TISSUE ENGINEERING

The Notch signaling network is evolutionarily conserved and has been described in a diversity of metazoans (10, 14-19). The Notch receptor (4 isoforms

identified in mammals) is initially synthesized as a single, 300kD precursor protein (20). This immature form undergoes furin-like proteolytic processing prior to its presentation on the cell surface. As a mature heterodimer, Notch is comprised of two major protein segments. The first is a large extracellular segment that contains many tandemly arranged EGF-like repeats. The second Notch segment is smaller and consists of a short ectodomain, single-pass transmembranal domain and intracellular domain. Together, the two receptor subunits associate via calcium-dependent, non-covalent interactions within their extracellular regions.

Notch signaling is activated by interactions with specific membrane-bound ligands on the surface of neighboring cells (4, 18, 21). Consequently, direct cell-cell contact is typically required for Notch signaling. Multiple Notch ligands have been characterized in mammals, e.g. Delta-like1 (also called Delta1), Delta-like3, Delta-like4, Jagged1, and Jagged2. All Notch ligands, except Delta-like3, have been found to physically and functionally interact with the Notch receptors, while Delta-like3 has been shown to attenuate activation of Notch by other ligands (22).

Like the Notch receptor itself, the members of the Notch ligand family, DSL (Delta/Serrate/LAG-2), also possess EGF-like repeats in their extracellular domains. These repeats are important for Notch-ligand interactions, and can be affected by various site-specific, post-translational modifications. For example, fucosylation, via an *N*-acetylglucosaminyltransferase activity of the Fringe protein, is critical for receptor-ligand binding. Furthermore, ligand affinity (e.g. signal potentiation through Delta vs. Jagged) can be altered through Fringe addition of *N*-acetylglucosamine to *O*-fucose (23, 24).

Upon ligand binding, Notch receptor undergoes proteolytic cleavage in both the extracellular and intracellular regions. The first cleavage is carried out by the ADAM family metalloprotease TACE (Tumor Necrosis Factor Alpha Converting Enzyme), and occurs immediately outside the cell membrane, releasing the entire ligand-bound extracellular portion of Notch. This fragment is subsequently endocytosed by the opposing ligand-presenting cell. In the second cleavage, the release of the Notch intracellular domain (NICD) is mediated by the action of presenilin-1-dependent gamma-secretase activity (20, 25). NICD contains nuclear localization signals and consequently undergoes nuclear translocation, where it binds to the major Notch effector – transcription factor CSL (CBF1 – humans, Suppressor of Hairless – Drosophila, LAG-1 – C. elegans, also termed RBP-J in mice) (26). It is important to note that, although poorly understood, CSL-independent Notch signaling events have also been described (27, 28).

Upon CSL binding, NICD promotes the displacement of a CSL-dependent transcriptional corepressor complex, which commonly includes a histone deacetylase and various corepressor components (29). NICD therefore behaves as a transcriptional co-activator, releasing DNA-bound CSL from its normal state of transcriptional repression to activation. Additionally, the displacement of CSL facilitates the recruitment of a transcriptional coactivation complex, including Mastermind/LAG-3/SEL-8 (30-33), thereby driving Notch target gene expression.

Despite the number of identified DNA-binding sites, the best characterized Notch target genes are basic helix-loop-helix (bHLH) proteins, e.g. HES – *Hairy/E(spl)* and (HESR/HEY) – HES-related family genes in vertebrates (34, 35). These proteins have been described as negative transcriptional regulators for a variety of genes involved in tissue-specific differentiation, cell cycle regulation and T-cell development (20, 23). Thus, although the specific identities of Notch target genes remain largely unresolved, it is clear that bHLH upregulation is an important feature of Notch transcriptional output.

Various manipulations of the signaling strength of this pathway, such as the forced ectopic expression of NICD and of Notch ligands, modulation of gamma-secretase activity and targeting of CSL, have been attempted experimentally and have yielded important information about the effects of the Notch signaling network on a variety of cell responses. These include data on somitogenesis, myogenesis, neurogenesis, and the transdifferentiation of bone marrow mesenchymal stem cells into muscle cells (10, 11, 21, 36, 37). Importantly, from a bioengineering perspective, many promising bio-pharmaceuticals targeting the Notch signaling pathway have been developed (38), and could be used in various tissue engineering methods as well as in the creation of novel synthetic materials that adopt Notch activity in *ex vivo* morphogenesis (discussed below).

3.1. General mechanism of action, a tissue engineering perspective

Described in general terms, the principal role of the Notch signaling pathway is to coordinate distinct cell fate determination in adjacent stem and progenitor cells. Some of the fundamental and classical mechanisms governing cell commitment to specific lineages, namely inductive interactions and lateral specification, are regulated by the Notch signaling network. According to a current paradigm experimentally established and best described in invertebrates (e.g. in flies and worms), Notch/LIN12 represent the molecular mechanisms by which uncommitted neighboring stem and progenitor cells instruct each other with respect to specific distinct cell fates (4, 14, 39-41). In the case of inductive interactions, cells are nonequivalent. For example, only one of the neighboring cells is induced to express the Notch ligand, thus activating Notch signaling in the adjacent cell but not in distant cells (4, 14, 42, 43). In contrast, lateral specification generates cells of different lineages from a population of equivalent cells through stochastic variation in the expression levels of Notch ligands and receptors. These variations are initially small but are amplified by multiple feedback mechanisms, which lead to instructive interactions between these cells and subsequent amplification of the differences in their cell-fate decisions (4, 14).

From a tissue engineering perspective, in lateral specification a gradual network of instructive signals develops in an initially non-committed, uniform group of

cells (44, 45). The re-creation of such a regulatory network is a tempting possibility for tissue engineering applications, where the initial populations of stem and progenitor cells are non-committed to a particular lineage, and their tissue-specific differentiation is the desired outcome. Despite the importance of creating an instructive synthetic environment for the deliberate manipulation of cell fate in tissue engineering approaches (46-48), a patterned asymmetry of Notch ligands, provided in a synthetic extracellular niche, might provide for the juxtaposition of cells with differential activity in Notch signaling. This difference would potentially utilize the natural feedback mechanisms for generating distinct cell fate determination from the initially equivalent uniform population of stem and progenitor cells. Thus, a non-equivalent patterning of Notch functional domains may advance current strategies for *ex vivo* tissue formation.

In this respect, initial studies have described the development of recombinantly-produced, chimeric extracellular matrix proteins that incorporate human Jagged1 and Delta1 functional domains into an elastin backbone (49). In a functionally-tested approach, an immobilized Notch ligand (Jagged 1-Fc fusion protein) was either coated onto the surface of polystyrene plates using affinity interactions with protein G, or was immobilized in poly-2-hydroxyethyl methacrylate (poly-HEMA), using affinity interactions with crosslinked rabbit anti-human Fc antibody (50). In this work, Jagged1, evenly distributed and oriented in its active conformation by affinity immobilization, predictably activated Notch signaling, and directed the expected differentiation of esophageal epithelial cells (50). Soluble Jagged1 did not have these effects, which is anticipated from earlier studies demonstrating that soluble Notch ligands act as antagonists of the pathway (51). In conclusion, future work employing creative choices of structural biomaterials, by examining cell types with high pluripotency and engineering an asymmetric pattern of immobilized Notch ligands (as opposed to the uniform distribution of these molecules), will aid these initial and important studies further. Additionally, such future developments will help ascertain whether a predictable cell-fate determination can be orchestrated from initially equivalent cell populations in synthetic micro-environments, and furthermore, if such deliberate lineage formation could produce synthetic tissues *ex vivo*.

Another aspect of Notch-exerted regulation that is interesting for tissue engineering applications concerns the fact that the Notch pathway is responsible for the formation of sharp and proper borderlines between the cells committed to different lineages (thus not only contributing to the formation of tissues, but also to organizing tissues into organ systems). This effect of Notch signaling is conserved in such evolutionary distinct species as *D. melanogaster* (52), mice and *Xenopus* (11). Specifically, activity of the Notch pathway controls formation of the dorsal–ventral boundary in fly wings (11), whereas in vertebrate species Notch is critically required for segmentation during somitogenesis (10, 11). Very interestingly, the robust effect of Notch on the segmentation of presomitic mesoderm (PSM) has been recently used to further scientific discovery with the help of real-time bioluminescence imaging methods (53). This particular work established that oscillation of the Notch effector, Hes-1, occurs in the PSM, but not in the individual PSM-derived cells. Furthermore, mathematical simulation suggests that Notch-controlled cell-cell communication is essential for the stability of cellular oscillators during segment formation (53).

Additionally, Notch signaling regulates the formation of anterior and posterior edges at the boundary between the dorsal and ventral thalamus in the developing avian brain (54). Notch also controls boundary formation in the zebrafish hindbrain (55). The effects of Notch signaling on the creation of tissue borders is linked to the activity of Fringe genes (e.g. lunatic-fringe in vertebrates), which possess the ability to modify the extracellular portion of Notch receptors (11, 56-58). Such modifications modulate the sensitivity of Notch receptors for ligands with differential binding affinity (e.g. Delta versus Serrate in *D. melanogaster,* or Delta versus Jagged in mammals (24, 59, 60)); and hence, fine-tune the signaling intensity of Notch (11, 24, 61).

A variety of well-developed bioengineering methods are perfectly suited for the *ex vivo* and *in situ* re-creation of precise borderlines between cells directed toward distinct fates by a patterned modulation of Notch signaling, thereby facilitating the procession from tissue to organ fabrication. For example, biomaterial researchers have coined the term "precursor tissue analog" (PTA), which generally implies a use of patterned biocompatible surfaces or three-dimensional structures for fabrication of functional tissues (62). The idea behind precursor tissue is that the engineered pattern is expected to promote physiological cell-cell interactions, leading to normal tissue remodeling by exposure of seeded cells to various signals patterned (62). The use of PTA for directed and specified differentiation of uncommitted stem and progenitor cells has also been envisioned. However, at this point, mostly patterning of natural adhesion molecules, cells, recombinant DNA molecules, and various growth factors have been approached experimentally (46, 48, 62-68).

One type of bioengineering technique that is particularly well-suited for delivering a tunable, temporal-spatial activation of patterned Notch are soft lithographic methods, such as microcontact patterning (46, 69). Discovered in microelectronics (69), microcontact patterning has recently flourished in biological applications (46, 70). This method is exemplified by the printing of avidin on poly-lactid–poly(ethylene glycol) (PLA–PEG) films (71) and by glass-surface printed protein A (72). Both techniques allow patterns of recombinant proteins of choice that are based on affinity interactions between avidin and biotinylated protein in the first case, and between protein A (or G) and the Fc fragment of immunoglobulin (IgG) produced as a fusion protein in the second case (71, 72). Importantly, the functional relevance of such patterned substrates has been proven by examining the effects of the axonal guidance molecule L1-Fc fusion protein on selective axon outgrowth when cells were cultured on patterned poy-L-lysine coated surfaces (72). Furthermore, the use of specific adhesion extra-cellular matrix substrates as a printing ink, allows optimization of these techniques for specific cell types, e.g. neural, endothelial, epithelial, etc (73-78). Application of these methods to temporal and spatial patterning of Notch ligands with differential receptor affinity may provide a unique method to direct the formation of

defined borderlines between cells committed to distinct lineages, which could be the first important step toward the dynamic process of *ex vivo* morphogenesis.

4. ROLE OF GRADIENTS, MODIFIERS AND INTERACTIONS WITH OTHER SIGNALING PATHWAYS IN NOTCH DIRECTED CELL-FATE DETERMINATION: CONSIDERATIONS FOR TISSUE ENGINEERING

Similar to other determinant factors regulating embryonic organogenesis, Notch signaling has morphogenic properties. Hence, cellular activity is controlled not simply by whether the Notch pathway is "on" or "off", but by the quantitatively variable magnitude and duration of Notch activity. Genetic targeting and conditional mutagenesis experiments in vertebrates have demonstrated that the quantity of Notch signaling activity is strictly controlled and is critically required for body patterning in early embryonic development, as well as for the establishment of specific organ systems, such as cardio-vascular and neural (79-86).

Importantly for bioengineering applications, very small quantitative differences in the degree of Notch signaling intensity translate into significant changes in functional cellular responses, ranging from proliferation to differentiation and from cell expansion to apoptosis (4, 5, 87). To further complicate this aspect, Notch is likely to control cell cycle progression both positively and negatively, in response to both intrinsic and extrinsic factors – depending on the strength of signaling and the molecular interactions of Notch signaling with other regulatory signal transduction networks (88-97). Therefore, either inducing or inhibiting Notch signaling by means of patterned synthetic material does not predictably control cell-fate determination, that is unless the specific biological context of such manipulations are also equally considered.

Physiologically, the range of Notch signaling intensity is regulated by many diverse positive and negative feed-back mechanisms, such as endocytosis, trafficking, and compartmentalization of Notch receptors and ligands. The strength of this signaling can be modulated by the attenuation of expression levels of Notch ligands in cells with active Notch, as well as by asymmetric localization or enhanced expression of Notch antagonists (i.e. Numb) (4, 11, 14, 15, 52, 98-106).

It is quite clear that in synthetic biomaterials aimed to re-create *ex vivo* morphogenesis, not only the presence and patterning of Notch-inducing and inhibitory elements, but also the controlled quantities or gradients of these elements should be considered. An attractive bioengineering method for generating a simple complex gradient of soluble factors, and delivering these factors in precise quantities, is microfluidics (107). Specifically, either a single or multiple stream could be used in a microfluidics device to deliver various signaling molecules in various combinations to cells. In such a system, both the concentration and the gradient of each molecule could be precisely controlled. Importantly, modern microcontact patterning and microfluidics methods either alone or in combination have been successfully used for generating gradients of signaling molecules. These experiments have primarily involved adhesion molecules (e.g. RGD (Arg–Gly–Asp) peptide), growth factors, or various densities of substrate as either transient or stable gradients (46, 107-110). The use of microfluidics, in combination with photopolymerization of monomers that gel by exposure to UV light, have been shown to produce patterned gradients of adhesion molecules in biomaterials (e.g. hydrogels) and have been proven to be functionally significant for the predictable manipulation of cell responses (111, 112). Several key cell responses, such as proliferation, differentiation, spreading, migration and natural matrix remodeling have been demonstrated to be controlled by the gradient density of adhesion ligands, e.g. RGD, and by the substrate density generated in synthetic hydrogels. In addition, microfuidic-orchestrated gradients of cytokines and growth factors have been used to study neutrophil chemotaxis, axon outgrowth and neurogenic differentiation of cells in culture (112-117). Therefore, formation of gradients of natural and synthetic Notch agonists and antagonists in engineered biomaterials is a real possibility. Combined with the appropriate positioning of stem and progenitor cells, these methods could be applied to the fine-tuning of directed *ex vivo* tissue and organ formation.

A final consideration for the use of the Notch signaling pathway in bioengineering applications involves its role as a master regulatory switch of cell-fate determination. In this manner, the Notch pathway interacts with all of the other networks known to control tissue formation and organogenesis in metazoans. Namely, Notch cross-talk with the Receptor Tyrosine Kinase-Ras-MAPK pathway, Jak-STAT signaling, the TGF-beta superfamily, wingless/Wnt and hedgehog/Shh pathways have been established in invertebrates and vertebrates, and have been found to be well conserved with respect to molecular mechanisms and the effects on cell behavior (5, 36, 118, 119). For example, it has been shown that Notch promotes the differentiation of astrocytes in the embryonic nervous system by activating STAT3 via facilitating complex formation between JAK2 and STAT3 (119). Notch has also been shown to interact with the Wnt pathway during the control of embryonic somitogenesis, hematopoiesis, and intestinal and skin tissue regeneration (120-123). Additionally, Notch and TGF-beta/phospho-Smad signaling interactions are important for embryonic organogenesis and for tissue regeneration in the adult, as well as for endothelial cell migration. Deregulation of this cross-talk has been shown to lead to mammary tumors (120, 124-128).

Therefore, changing the activity of Notch in synthetic scaffolds will likely change the signal transduction of many other physiologically important networks in the cells of interest, where specific changes will depend on the strength of signaling and the cell types involved. In contrast, manipulating the activity of signaling networks, such as Shh, TGF-beta or Tyrosine-Kinase-Ras-MAPK, which is one of the common results of biomaterial-enabled patterned release of BMP ligands and growth factors, (e.g VEGF) (48, 63, 66, 129-132) will very likely alter the strength of Notch signaling in cells exposed to such synthetic scaffolds. The issue of signal integration and the specific cellular and molecular context in which the Notch pathway is manipulated should definitely be

taken into account. As such, the cell-fate decisions and the final effect on tissue fabrication will result not only from the modification of the Notch signaling pathway, but from the simultaneous changes in other signaling networks cumulatively controlling cellular responses.

A specific and classical example Notch and Ras pathway interaction in regulating cell fate determination has been established in invertebrate embryogenesis, where the formation of *C. elegans* vulva and the *Drosophila* ommatidia-eye are controlled by the cross-talk between these signaling pathways (5, 133-136). Importantly, the actual mechanism of cell fate determination regulation by Notch and Ras interplay are well conserved between invertebrates and mammals, as well as between different tissues (137-144). Described in general terms, Notch-imposed lateral specification creates asymmetry in a cluster of cells with respect to the expression of a specific growth factor, such as EGF. This asymmetry, in turn, creates a gradient of tyrosine kinase receptor-Ras activation in such cell populations. Since Ras signaling induces the expression of Notch ligands, the differential Ras activation is translated into instructive Notch signaling, hence generating distinct cell fates and leading to tissue/organ formation (5).

Several tissue engineering approaches for, signal integration and for simultaneous delivery of multiple growth factors have been described, and are well suited for creating a proper molecular context for manipulations of the Notch pathway (108, 145-148). For example, mature vascular network formation has been accomplished by dual delivery of vascular endothelial growth factor and platelet-derived growth factor from a single, structural (PLG) polymer scaffold (108). Similarly, biodegradable hydrogels have been used for simultaneous delivery of insulin-like growth factor-1 and transforming growth factor-beta1 into injured cartilage (145). Hydrogels have also enabled the simultaneous delivery of bone morphogenetic protein-2 and transforming growth factor-beta3 to transplanted bone marrow stromal cells, which significantly improved the regenerative outcome (146). In a microfluidic approach, multiple laminar streams have been used in a microfluidic channel to deliver membrane-permeable molecules, such as flourescent tags to selected subcellular microdomains of single cells (147). It is easy to envision that the use of such methods could create complex soluble niches by positioning cells at an interface between several laminar streams, thereby delivering multiple signaling molecules that could also be asymmetrically organized with respect to their gradients (46, 107, 147).

5. NOTCH SIGNALING IN MAINTENANCE AND REPAIR OF VARIOUS ADULT TISSUES, AND THE BIOENGINEERING APPROACHES FOR MANIPULATION OF NOTCH SIGNALING DURING TISSUE REGENERATION

An emerging paradigm is that conserved signaling pathways, such as Notch, not only regulate the formation of organs during embryonic development, but are also employed for postnatal organ repair. Consequentially, the deregulation of these key determinants of cell-fate determination cause not only developmental abnormalities, but also result in a variety of adult disorders (10, 11, 19, 21, 36, 149). The role of Notch in the maintenance and regeneration of postembryonic tissues, and the disorders associated with the deregulation of Notch activity are discussed below. Also, the bioengineering approaches for manipulation of Notch signaling during tissue regeneration are introduced.

5.1. The role of Notch signaling in the maintenance and repair of healthy tissues

While Notch-Delta signaling has been extensively studied in regard to development and organogenesis, the role of Notch in the maintenance and repair of various adult tissues is only beginning to be elucidated. The precise role that Notch plays in stem cell biology varies from tissue to tissue and from cell type to cell type, but general themes are emerging as more tissues are studied. In many cases, Notch signaling promotes proliferation and homeostasis over differentiation, and has also been shown to regulate apoptosis (11, 21, 36).

Conboy et al. demonstrated that signaling through the Notch pathway is essential for maintenance of muscle tissue (150). Following injury, Notch signaling must be upregulated for efficient activation of satellite cells (muscle stem cells). Specifically, expression of the Notch ligand Delta is upregulated in response to injury. Likewise, the inhibition of Notch signaling was found to dramatically decrease the efficiency of muscle wound healing (151). This response also seems to be a major factor in muscle aging, as aged muscle has a diminished ability to respond to injury and fails to upregulate Delta in response to injury. Furthermore, efficient muscle healing could be rescued through direct activation of Notch *in vivo*.

Like muscle, neuronal tissue is primarily post-mitotic. However, this aspect alone does not equate to clear similarities in the Notch signaling behavior within these two tissue types. There are many different types of neural cells, and so the picture is somewhat more complicated. For example, Notch signaling promotes the differentiation of astrocytes, radial glia, and Müller cells (119, 152-159). In terms of the self-renewing neural stem cells (NSC), however, Notch activity seems to follow the conserved route of promoting maintenance and suppressing differentiation. In animals deficient for the Notch effector Hes1, the brain NSC population is greatly diminished (160). Furthermore, modulation of Notch signaling in neural stem cell cultures can strictly regulate the decision between self renewal and differentiation (161).

Notch inhibits oligodendrocyte differentiation, but promotes the differentiation of astrocyte, radial glia and Müller cells in retina formation. However, in the epidermis of the skin, Notch signaling actually promotes differentiation of hair follicles, whereas Notch inhibition causes proliferation (162).

The intestine is an excellent system to study stem cells and their role in the everyday maintenance of a tissue. In the intestine, the stem cells reside near the base of the crypts of Lieberkühn (163). It is there that all four distinct cell types of the intestine are derived from the resident stem cells. New intestinal cells are continuously produced to replace the

sloughing off of cells from the tips of the villi. In this respect, simultaneous activation of both Notch and Wnt signaling pathways is necessary for the self renewal of intestinal stem cells (162). Wnt signaling occurs almost exclusively in the crypts, and Notch/Delta expression is further confined to the base of the crypts (163) . Lastly, intestinal Wnt signaling is sufficient to activate Notch and stimulate proliferation.

Hematopoietic stem cells are similarly regulated by Wnt and Notch (164-166). Wnt expression activates Notch and in turn signals for self renewal (121). It seems likely that presentation of Notch ligands in the bone marrow niche is a signal through which stemness is preserved. However, while Notch activation does prevent B-cell differentiation, Notch has been shown to promote the T-cell lineage (167-169).

Thus far, the role of Notch signaling seems best understood where it has been studied in the gut and in the skeletal muscle systems. Other systems, particularly the hematopoietic and nervous systems are considerably more complicated and warrant further study. Of particular perplexity is how and why the Notch/Delta signaling family seems to force proliferation in most cases, while certain other cell types choose exactly the opposite cell fate decision.

5.2. Disorders and abnormalities associated with the deregulation of Notch signlaling

As an established oncogene in the hematopoietic system in humans, Notch1 receptor has been implicated in T-cell acute lymphoblastic leukemia (T-ALL). Chromosomal translocation events in T-ALL result in the constitutive activation of a truncated Notch1 form, referred to as TAN1 (translocation-associated Notch homologue) (170). As such, constitutively active NICD appears to be largely responsible for the Notch-related T-cell malignancies in hematopoietic stem cells. Recent work with mouse models suggests that the role of Notch in T-cell receptor recombination events may be critical to the development of T-ALL (171), and, in one study, involved mutations within domains affecting receptor stability (172). Interestingly, in addition to Notch1, other aberrant forms of this receptor have been reported to similarly induce T-cell leukemia in committed thymocyte progenitors (173).

Mutation in the Notch pathway components have also been implicated in several inheritable human diseases, such as Alagille Syndrome (AGS), CADASIL (Cerebral Autosomal Dominant Arteriopathy with Sub-cortical Infarcts and Leukoencephalopathy) and Spondylocostal dysostosis (SD) (174). In a large number of AGS patients, Jagged1 expression is diminished as a result of mutations, e.g. premature termination codons within the gene or abnormal glycosylation causing diminished cell surface transport-expression of Jagged1 protein (174-177). Genetic and environmental modifiers have been suggested to explain apparent variance in AGS pathology, within patients carrying identical JAG1 mutations (178-180). In this respect, advances in the development of AGS mouse models, such as Hey2 homozygous mutants suggest that genes encoding other Notch signaling pathway components are likely candidates (80, 181, 182).

Inherited forms of autosomal-recessive SD were demonstrated to be caused by mutations in the modifiers of Notch signaling strength, e.g. Delta-like3 and Lunatic fringe (183-187). The phenotype for SD is very similar to that exhibited by Dll3pu mutant mice (homozygous for pudgy mutation) and by Lfng homozygous null mutations, implicating the loss of function of these genes in human SD (183, 185-188).

Mutations in the Notch3 gene are attributed to causing CADASIL (189), and involve a gain or loss of one of the six conserved cysteine residues that comprise the EGF-like repeats of the Notch receptor extracellular domain. It is currently unknown how Notch3 signaling is altered by CADASIL mutations, as they do not appear to directly affect cell surface expression or ligand binding (190). However, correlations between human patients with CADASIL and the mouse CADASIL model, where Notch3 ectodomain has been shown to accumulate in cerebral and peripheral arterioles, suggests that CADASIL mutations may compromise Notch3 ectodomain clearance, following ligand interaction (191, 192).

In summary, the Notch signaling pathway plays a pivotal role in the maintenance and repair of multiple adult tissues and many disorders, as well as the age-related decline in tissue regenerative potential stem from the deregulation of this signaling network. Therefore, important therapeutic applications could be developed to enhance the regeneration of organ repair, especially in the elderly and those afflicted by degenerative disorders. Novel therapies for Notch-associated genetic diseases and oncogenic transformations could result from adapting some of the tissue engineering approaches described above for the deliberate manipulation of the Notch signaling pathway. One such approach, perfectly suited for the enhancement of tissue repair and for gene-therapy applications, is acellular therapy, which involves the introduction of cell-free biodegradable materials composed of natural and synthetic extra-cellular matrix components, capable of releasing biologically active molecules in the tissue of interest, *in situ* (48, 63, 193, 194). Moreover, an interactive factor delivery method could be orchestrated from synthetic biomaterials, where endogenous cells activate the factor by local secretion of proteases and, not only single, but multiple factors could be released simultaneously or sequentially (108, 129, 195-200). Functional ECM analogs, such as hydrogels with controlled chemical and biological properties and abilities to influence regenerative responses have already been developed and tested in science and medicine (64-66, 201-205). Adapting these principles for designing synthetic materials, able to control the signaling intensity of Notch *in situ*, could be based on an integration of specific physiological or synthetic biologically active modulators of this pathway (described above). Positive natural modulators, such as Notch ligands, could be used for enhancing Notch activity in cases of age-related decline in tissue repair and in some genetic disorders. In contrast, negative modifiers, such as inhibitors of gamma-secretase or recombinant Notch antagonists, consisting of ligand-binding EGF repeats, and thus, serving as a “sponge” or

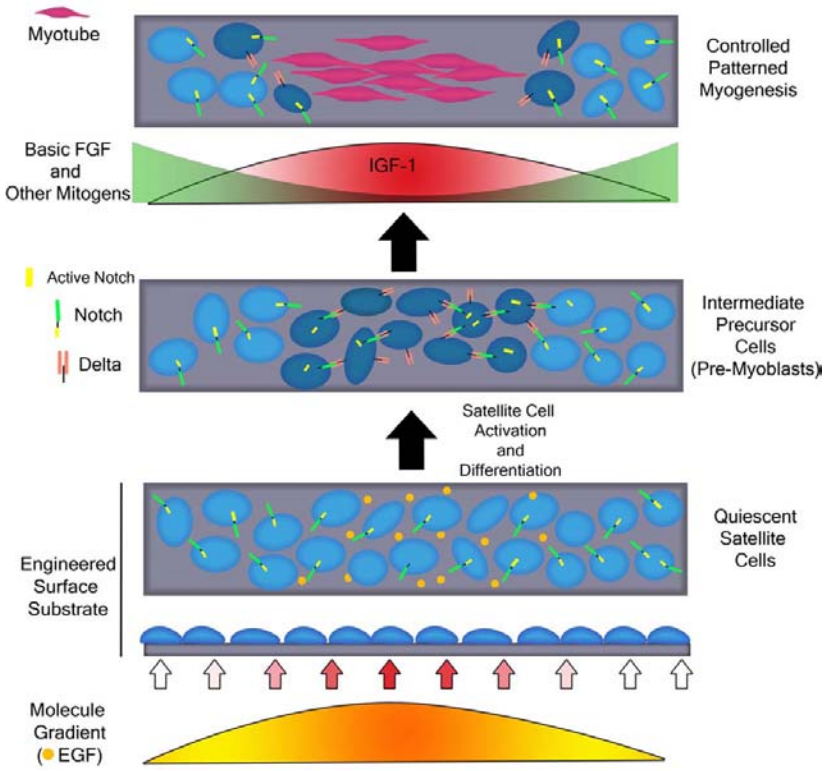

Figure 1. Hypothetical synthetic scaffold adapting Notch signaling for *ex vivo* myogenesis. Adult stem cells such as quiescent satellite cells (muscle stem cells expressing inactive Notch receptor) can be cultured on synthetic biomaterials (either in 2D or in 3D scaffolds), which release growth factors (i.e. EGF) in a gradient fashion and initiate asymmetric stem cell differentiation via variable Notch signaling. In this hypothetical scheme, muscle stem cells exposed to high levels of EGF begin to express Notch ligands (i.e. Delta) which can activate Notch in neighboring, but not distant cells. The highest concentration of EGF might also attenuate Notch (via Ras) in the cells that have been orchestrated to up-regulate Delta most efficiently, and such cells might be prone to differentiate into fusion-competent myoblasts. At the same time, cells exposed to moderate or low levels of EGF and adjacent to Delta-expressing neighbors, will have the highest level of Notch activation; and thus will be directed toward a highly proliferative intermediate precursor cell fate (pre-myoblasts) and will be resistant to terminal differentiation into myotubes. Hence, three populations of cells will be created from one initially equivalent cell group: original satellite cells at the periphery, cells with high levels of Delta but modest Notch activation in the center, and intermediate progenitor cells with high Notch activity in between. Subsequently, mitogenic growth factors, such as basic FGF (bFGF) can be withdrawn and replaced with differentiation-promoting factors, e.g IGF-1, thus forming the second gradient that directs terminal differentiation of muscle progenitor cells into fused myotubes. Importantly, muscle stem and progenitor cells can be manipulated to co-exist with the differentiated muscle cells, thus recreating dynamically remodeling living tissue by controlling variable cell fate determination on a single substrate, through a gradient-based asymmetry in Notch signaling strength.

"decoy" for natural ligands could be used to treat malignancies (4, 25, 38).

6. CONCLUDING REMARKS

The Notch signaling pathway has been extensively investigated in many organisms and by using many experimental systems for ~ 90 years, thus it is truly remarkable that up-to-date studies of this pathway continue to shed light on the conserved molecular mechanisms by which cells communicate with each other, commit to a specific cell-fate and form organs and tissues. The virtually universal ability of the Notch pathway to control cell behavior during embryonic development and in the adult, in combination with the reasonably well understood Notch-specific signal transduction events, makes this network very adaptable for tissue engineering applications. Importantly, the bioengineering components that are necessary for the deliberate manipulation of Notch signaling are already in place, e.g. smart biomaterials able to pattern Notch ligands or create gradients of Notch agonists and antagonists. Positioning initially equivalent cell populations in materials possessing asymmetric properties, with respect to the induction of Notch activity, has a great potential for *ex vivo* morphogenesis or for creating dynamically regenerating adult tissues composed of stem, progenitor and differentiated cell subsets derived from one cell-fate uniform population. As an example of future avenues that utilize Notch signaling pathway in tissue engineering, we depict a hypothetical *ex vivo* adult myogenesis, orchestrated and controlled by gradient-based manipulations of Notch signaling, Figure 1.

7. REFERENCES

1. Dexter, G.: The analysis of a case of continuous variation in Drosophila by a study of its linkage relation. *Am Nat*, 48, 712 - 758 (1914)
2. Morgan, T. H. & C. B. Bridges: Sex-linked inheritance in Drosophila. *Carnegie Inst Washington Publ*, 237, 87 (1916)
3. Mohr, O. L.: Character changes caused by mutation of an entire region of a chromosome in Drosophila. *Genetics*, 4, 275 - 282 (1919)
4. Louvi, A. & S. Artavanis-Tsakonas: Notch signalling in vertebrate neural development. *Nat Rev Neurosci*, 7, 93-102 (2006)
5. Sundaram, M. V.: The love-hate relationship between Ras and Notch. *Genes Dev*, 19, 1825-39 (2005)
6. Conlon, R. A., A. G. Reaume & J. Rossant: Notch1 is required for the coordinate segmentation of somites. *Development*, 121, 1533-45 (1995)
7. Hrabe de Angelis, M., J. McIntyre, 2nd & A. Gossler: Maintenance of somite borders in mice requires the Delta homologue DII1. *Nature*, 386, 717-21 (1997)
8. Gridley, T.: Notch signaling in vertebrate development and disease. *Mol Cell Neurosci*, 9, 103-8 (1997)
9. Weinmaster, G., V. J. Roberts & G. Lemke: A homolog of Drosophila Notch expressed during mammalian development. *Development*, 113, 199-205 (1991)
10. Weinmaster, G.: The ins and outs of notch signaling. *Mol Cell Neurosci*, 9, 91-102 (1997)
11. Weinmaster, G. & C. Kintner: Modulation of notch signaling during somitogenesis. *Annu Rev Cell Dev Biol*, 19, 367-95 (2003)
12. Chitnis, A. B.: The role of Notch in lateral inhibition and cell fate specification. *Mol Cell Neurosci*, 6, 311-21 (1995)
13. de la Pompa, J. L., A. Wakeham, K. M. Correia, E. Samper, S. Brown, R. J. Aguilera, T. Nakano, T. Honjo, T. W. Mak, J. Rossant & R. A. Conlon: Conservation of the Notch signalling pathway in mammalian neurogenesis. *Development*, 124, 1139-48 (1997)
14. Greenwald, I.: LIN-12/Notch signaling: lessons from worms and flies. *Genes Dev*, 12, 1751-62 (1998)
15. Artavanis-Tsakonas, S., M. D. Rand & R. J. Lake: Notch signaling: cell fate control and signal integration in development. *Science*, 284, 770-6 (1999)
16. Kadesch, T.: Notch signaling: the demise of elegant simplicity. *Curr Opin Genet Dev*, 14, 506-12 (2004)
17. Kimble, J. & P. Simpson: The LIN-12/Notch signaling pathway and its regulation. *Annu Rev Cell Dev Biol*, 13, 333-61 (1997)
18. Egan, S. E., B. St-Pierre & C. C. Leow: Notch receptors, partners and regulators: from conserved domains to powerful functions. *Curr Top Microbiol Immunol*, 228, 273-324 (1998)
19. Gridley, T.: Notch signaling and inherited disease syndromes. *Hum Mol Genet*, 12 Spec No 1, R9-13 (2003)
20. Radtke, F., A. Wilson, S. J. Mancini & H. R. MacDonald: Notch regulation of lymphocyte development and function. *Nat Immunol*, 5, 247-53 (2004)
21. Chiba, S.: Notch signaling in stem cell systems. *Stem Cells* (2006)
22. Ladi, E., J. T. Nichols, W. Ge, A. Miyamoto, C. Yao, L. T. Yang, J. Boulter, Y. E. Sun, C. Kintner & G. Weinmaster: The divergent DSL ligand Dll3 does not activate Notch signaling but cell autonomously attenuates signaling induced by other DSL ligands. *J Cell Biol*, 170, 983-92 (2005)
23. Ishibashi, M.: Molecular mechanisms for morphogenesis of the central nervous system in mammals. *Anat Sci Int*, 79, 226-34 (2004)
24. Haines, N. & K. D. Irvine: Glycosylation regulates Notch signalling. *Nat Rev Mol Cell Biol*, 4, 786-97 (2003)
25. Fortini, M. E.: Gamma-secretase-mediated proteolysis in cell-surface-receptor signalling. *Nat Rev Mol Cell Biol*, 3, 673-84 (2002)
26. Lai, E. C.: Keeping a good pathway down: transcriptional repression of Notch pathway target genes by CSL proteins. *EMBO Rep*, 3, 840-5 (2002)
27. Brennan, K. & P. Gardner: Notching up another pathway. *Bioessays*, 24, 405-10 (2002)
28. Martinez Arias, A., V. Zecchini & K. Brennan: CSL-independent Notch signalling: a checkpoint in cell fate decisions during development? *Curr Opin Genet Dev*, 12, 524-33 (2002)
29. Kao, H. Y., P. Ordentlich, N. Koyano-Nakagawa, Z. Tang, M. Downes, C. R. Kintner, R. M. Evans & T. Kadesch: A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. *Genes Dev*, 12, 2269-77 (1998)
30. Doyle, T. G., C. Wen & I. Greenwald: SEL-8, a nuclear protein required for LIN-12 and GLP-1 signaling in Caenorhabditis elegans. *Proc Natl Acad Sci U S A*, 97, 7877-81 (2000)
31. Petcherski, A. G. & J. Kimble: Mastermind is a putative activator for Notch. *Curr Biol*, 10, R471-3 (2000)
32. Wu, L., J. C. Aster, S. C. Blacklow, R. Lake, S. Artavanis-Tsakonas & J. D. Griffin: MAML1, a human homologue of Drosophila mastermind, is a transcriptional co-activator for NOTCH receptors. *Nat Genet*, 26, 484-9 (2000)
33. Kitagawa, M., T. Oyama, T. Kawashima, B. Yedvobnick, A. Kumar, K. Matsuno & K. Harigaya: A human protein with sequence similarity to Drosophila mastermind coordinates the nuclear form of notch and a CSL protein to build a transcriptional activator complex on target promoters. *Mol Cell Biol*, 21, 4337-46 (2001)
34. Bailey, A. M. & J. W. Posakony: Suppressor of hairless directly activates transcription of enhancer of split complex genes in response to Notch receptor activity. *Genes Dev*, 9, 2609-22 (1995)
35. Iso, T., L. Kedes & Y. Hamamori: HES and HERP families: multiple effectors of the Notch signaling pathway. *J Cell Physiol*, 194, 237-55 (2003)
36. Molofsky, A. V., R. Pardal & S. J. Morrison: Diverse mechanisms regulate stem cell self-renewal. *Curr Opin Cell Biol*, 16, 700-7 (2004)
37. Dezawa, M., H. Ishikawa, Y. Itokazu, T. Yoshihara, M. Hoshino, S. Takeda, C. Ide & Y. Nabeshima: Bone marrow stromal cells generate muscle cells and repair muscle degeneration. *Science*, 309, 314-7 (2005)
38. Zlobin, A., M. Jang & L. Miele: Toward the rational design of cell fate modifiers: notch signaling as a target for

novel biopharmaceuticals. *Curr Pharm Biotechnol*, 1, 83-106 (2000)
39. Mango, S. E., C. J. Thorpe, P. R. Martin, S. H. Chamberlain & B. Bowerman: Two maternal genes, apx-1 and pie-1, are required to distinguish the fates of equivalent blastomeres in the early Caenorhabditis elegans embryo. *Development*, 120, 2305-15 (1994)
40. Mello, C. C., B. W. Draper & J. R. Priess: The maternal genes apx-1 and glp-1 and establishment of dorsal-ventral polarity in the early C. elegans embryo. *Cell*, 77, 95-106 (1994)
41. Mickey, K. M., C. C. Mello, M. K. Montgomery, A. Fire & J. R. Priess: An inductive interaction in 4-cell stage C. elegans embryos involves APX-1 expression in the signalling cell. *Development*, 122, 1791-8 (1996)
42. Henderson, S. T., D. Gao, E. J. Lambie & J. Kimble: lag-2 may encode a signaling ligand for the GLP-1 and LIN-12 receptors of C. elegans. *Development*, 120, 2913-24 (1994)
43. Crittenden, S. L., E. R. Troemel, T. C. Evans & J. Kimble: GLP-1 is localized to the mitotic region of the C. elegans germ line. *Development*, 120, 2901-11 (1994)
44. Heitzler, P. & P. Simpson: The choice of cell fate in the epidermis of Drosophila. *Cell*, 64, 1083-92 (1991)
45. Parks, A. L., S. S. Huppert & M. A. Muskavitch: The dynamics of neurogenic signalling underlying bristle development in Drosophila melanogaster. *Mech Dev*, 63, 61-74 (1997)
46. Khademhosseini, A., R. Langer, J. Borenstein & J. P. Vacanti: Microscale technologies for tissue engineering and biology. *Proc Natl Acad Sci U S A*, 103, 2480-7 (2006)
47. Hubbell, J. A.: Materials as morphogenetic guides in tissue engineering. *Curr Opin Biotechnol*, 14, 551-8 (2003)
48. Rosso, F., G. Marino, A. Giordano, M. Barbarisi, D. Parmeggiani & A. Barbarisi: Smart materials as scaffolds for tissue engineering. *J Cell Physiol*, 203, 465-70 (2005)
49. Liu, C. Y., U. Westerlund, M. Svensson, M. C. Moe, M. Varghese, J. Berg-Johnsen, M. L. Apuzzo, D. A. Tirrell & I. A. Langmoen: Artificial niches for human adult neural stem cells: possibility for autologous transplantation therapy. *J Hematother Stem Cell Res*, 12, 689-99 (2003)
50. Beckstead, B. L., D. M. Santosa & C. M. Giachelli: Mimicking cell-cell interactions at the biomaterial-cell interface for control of stem cell differentiation. *J Biomed Mater Res A* (2006)
51. Hicks, C., E. Ladi, C. Lindsell, J. J. Hsieh, S. D. Hayward, A. Collazo & G. Weinmaster: A secreted Delta1-Fc fusion protein functions both as an activator and inhibitor of Notch1 signaling. *J Neurosci Res*, 68, 655-67 (2002)
52. Irvine, K. D.: Fringe, Notch, and making developmental boundaries. *Curr Opin Genet Dev*, 9, 434-41 (1999)
53. Masamizu, Y., T. Ohtsuka, Y. Takashima, H. Nagahara, Y. Takenaka, K. Yoshikawa, H. Okamura & R. Kageyama: Real-time imaging of the somite segmentation clock: revelation of unstable oscillators in the individual presomitic mesoderm cells. *Proc Natl Acad Sci U S A*, 103, 1313-8 (2006)
54. Zeltser, L. M., C. W. Larsen & A. Lumsden: A new developmental compartment in the forebrain regulated by Lunatic fringe. *Nat Neurosci*, 4, 683-4 (2001)
55. Pan, Y., M. H. Lin, X. Tian, H. T. Cheng, T. Gridley, J. Shen & R. Kopan: gamma-secretase functions through Notch signaling to maintain skin appendages but is not required for their patterning or initial morphogenesis. *Dev Cell*, 7, 731-43 (2004)
56. Munro, S. & M. Freeman: The notch signalling regulator fringe acts in the Golgi apparatus and requires the glycosyltransferase signature motif DXD. *Curr Biol*, 10, 813-20 (2000)
57. Hicks, C., S. H. Johnston, G. diSibio, A. Collazo, T. F. Vogt & G. Weinmaster: Fringe differentially modulates Jagged1 and Delta1 signalling through Notch1 and Notch2. *Nat Cell Biol*, 2, 515-20 (2000)
58. Panin, V. M., V. Papayannopoulos, R. Wilson & K. D. Irvine: Fringe modulates Notch-ligand interactions. *Nature*, 387, 908-12 (1997)
59. Jaleco, A. C., H. Neves, E. Hooijberg, P. Gameiro, N. Clode, M. Haury, D. Henrique & L. Parreira: Differential effects of Notch ligands Delta-1 and Jagged-1 in human lymphoid differentiation. *J Exp Med*, 194, 991-1002 (2001)
60. Lehar, S. M., J. Dooley, A. G. Farr & M. J. Bevan: Notch ligands Delta 1 and Jagged1 transmit distinct signals to T-cell precursors. *Blood*, 105, 1440-7 (2005)
61. Visan, I., J. B. Tan, J. S. Yuan, J. A. Harper, U. Koch & C. J. Guidos: Regulation of T lymphopoiesis by Notch1 and Lunatic fringe-mediated competition for intrathymic niches. *Nat Immunol*, 7, 634-43 (2006)
62. Nishimura, I., R. L. Garrell, M. Hedrick, K. Iida, S. Osher & B. Wu: Precursor tissue analogs as a tissue-engineering strategy. *Tissue Eng*, 9 Suppl 1, S77-89 (2003)
63. Lutolf, M. P. & J. A. Hubbell: Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotechnol*, 23, 47-55 (2005)
64. Chen, R. R. & D. J. Mooney: Polymeric growth factor delivery strategies for tissue engineering. *Pharm Res*, 20, 1103-12 (2003)
65. Boontheekul, T. & D. J. Mooney: Protein-based signaling systems in tissue engineering. *Curr Opin Biotechnol*, 14, 559-65 (2003)
66. Zisch, A. H., M. P. Lutolf & J. A. Hubbell: Biopolymeric delivery matrices for angiogenic growth factors. *Cardiovasc Pathol*, 12, 295-310 (2003)
67. Jang, J. H., T. L. Houchin & L. D. Shea: Gene delivery from polymer scaffolds for tissue engineering. *Expert Rev Med Devices*, 1, 127-38 (2004)
68. Jang, J. H., C. B. Rives & L. D. Shea: Plasmid delivery in vivo from porous tissue-engineering scaffolds: transgene expression and cellular transfection. *Mol Ther*, 12, 475-83 (2005)
69. Falconnet, D., G. Csucs, H. M. Grandin & M. Textor: Surface engineering approaches to micropattern surfaces for cell-based assays. *Biomaterials*, 27, 3044-63 (2006)
70. Singhvi, R., A. Kumar, G. P. Lopez, G. N. Stephanopoulos, D. I. Wang, G. M. Whitesides & D. E. Ingber: Engineering cell shape and function. *Science*, 264, 696-8 (1994)
71. Patel, N., R. Bhandari, K. M. Shakesheff, S. M. Cannizzaro, M. C. Davies, R. Langer, C. J. Roberts, S. J. Tendler & P. M. Williams: Printing patterns of biospecifically-adsorbed protein. *J Biomater Sci Polym Ed*, 11, 319-31 (2000)

72. Oliva, A. A., Jr., C. D. James, C. E. Kingman, H. G. Craighead & G. A. Banker: Patterning axonal guidance molecules using a novel strategy for microcontact printing. *Neurochem Res*, 28, 1639-48 (2003)
73. Kam, L. & S. G. Boxer: Cell adhesion to protein-micropatterned-supported lipid bilayer membranes. *J Biomed Mater Res*, 55, 487-95 (2001)
74. Csucs, G., R. Michel, J. W. Lussi, M. Textor & G. Danuser: Microcontact printing of novel co-polymers in combination with proteins for cell-biological applications. *Biomaterials*, 24, 1713-20 (2003)
75. Lehnert, D., B. Wehrle-Haller, C. David, U. Weiland, C. Ballestrem, B. A. Imhof & M. Bastmeyer: Cell behaviour on micropatterned substrata: limits of extracellular matrix geometry for spreading and adhesion. *J Cell Sci*, 117, 41-52 (2004)
76. Klein, C. L., M. Scholl & A. Maelicke: Neuronal networks in vitro: formation and organization on biofunctionalized surfaces. *J Mater Sci Mater Med*, 10, 721-7 (1999)
77. Kam, L., W. Shain, J. N. Turner & R. Bizios: Axonal outgrowth of hippocampal neurons on micro-scale networks of polylysine-conjugated laminin. *Biomaterials*, 22, 1049-54 (2001)
78. Cornish, T., D. W. Branch, B. C. Wheeler & J. T. Campanelli: Microcontact printing: a versatile technique for the study of synaptogenic molecules. *Mol Cell Neurosci*, 20, 140-53 (2002)
79. Donoviel, D. B., A. K. Hadjantonakis, M. Ikeda, H. Zheng, P. S. Hyslop & A. Bernstein: Mice lacking both presenilin genes exhibit early embryonic patterning defects. *Genes Dev*, 13, 2801-10 (1999)
80. McCright, B., J. Lozier & T. Gridley: A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. *Development*, 129, 1075-82 (2002)
81. Krebs, L. T., J. R. Shutter, K. Tanigaki, T. Honjo, K. L. Stark & T. Gridley: Haploinsufficient lethality and formation of arteriovenous malformations in Notch pathway mutants. *Genes Dev*, 18, 2469-73 (2004)
82. Duarte, A., M. Hirashima, R. Benedito, A. Trindade, P. Diniz, E. Bekman, L. Costa, D. Henrique & J. Rossant: Dosage-sensitive requirement for mouse Dll4 in artery development. *Genes Dev*, 18, 2474-8 (2004)
83. Gale, N. W., M. G. Dominguez, I. Noguera, L. Pan, V. Hughes, D. M. Valenzuela, A. J. Murphy, N. C. Adams, H. C. Lin, J. Holash, G. Thurston & G. D. Yancopoulos: Haploinsufficiency of delta-like 4 ligand results in embryonic lethality due to major defects in arterial and vascular development. *Proc Natl Acad Sci U S A*, 101, 15949-54 (2004)
84. Tomita, K., M. Ishibashi, K. Nakahara, S. L. Ang, S. Nakanishi, F. Guillemot & R. Kageyama: Mammalian hairy and Enhancer of split homolog 1 regulates differentiation of retinal neurons and is essential for eye morphogenesis. *Neuron*, 16, 723-34 (1996)
85. Fischer, A., N. Schumacher, M. Maier, M. Sendtner & M. Gessler: The Notch target genes Hey1 and Hey2 are required for embryonic vascular development. *Genes Dev*, 18, 901-11 (2004)
86. Petersen, P. H., K. Zou, J. K. Hwang, Y. N. Jan & W. Zhong: Progenitor cell maintenance requires numb and numblike during mouse neurogenesis. *Nature*, 419, 929-34 (2002)
87. Kiaris, H., K. Politi, L. M. Grimm, M. Szabolcs, P. Fisher, A. Efstratiadis & S. Artavanis-Tsakonas: Modulation of notch signaling elicits signature tumors and inhibits hras1-induced oncogenesis in the mouse mammary epithelium. *Am J Pathol*, 165, 695-705 (2004)
88. Go, M. J., D. S. Eastman & S. Artavanis-Tsakonas: Cell proliferation control by Notch signaling in Drosophila development. *Development*, 125, 2031-40 (1998)
89. de Celis, J. F., D. M. Tyler, J. de Celis & S. J. Bray: Notch signalling mediates segmentation of the Drosophila leg. *Development*, 125, 4617-26 (1998)
90. Diaz-Benjumea, F. J. & S. M. Cohen: Serrate signals through Notch to establish a Wingless-dependent organizer at the dorsal/ventral compartment boundary of the Drosophila wing. *Development*, 121, 4215-25 (1995)
91. Markopoulou, K. & S. Artavanis-Tsakonas: The expression of the neurogenic locus Notch during the postembryonic development of Drosophila melanogaster and its relationship to mitotic activity. *J Neurogenet*, 6, 11-26 (1989)
92. Moberg, K. H., S. Schelble, S. K. Burdick & I. K. Hariharan: Mutations in erupted, the Drosophila ortholog of mammalian tumor susceptibility gene 101, elicit non-cell-autonomous overgrowth. *Dev Cell*, 9, 699-710 (2005)
93. Thompson, B. J., J. Mathieu, H. H. Sung, E. Loeser, P. Rorth & S. M. Cohen: Tumor suppressor properties of the ESCRT-II complex component Vps25 in Drosophila. *Dev Cell*, 9, 711-20 (2005)
94. Baonza, A. & M. Freeman: Control of cell proliferation in the Drosophila eye by Notch signaling. *Dev Cell*, 8, 529-39 (2005)
95. Johnston, L. A. & B. A. Edgar: Wingless and Notch regulate cell-cycle arrest in the developing Drosophila wing. *Nature*, 394, 82-4 (1998)
96. Ohnuma, S., A. Philpott, K. Wang, C. E. Holt & W. A. Harris: p27Xic1, a Cdk inhibitor, promotes the determination of glial cells in Xenopus retina. *Cell*, 99, 499-510 (1999)
97. Ohnuma, S., S. Hopper, K. C. Wang, A. Philpott & W. A. Harris: Co-ordinating retinal histogenesis: early cell cycle exit enhances early cell fate determination in the Xenopus retina. *Development*, 129, 2435-46 (2002)
98. Le Borgne, R., A. Bardin & F. Schweisguth: The roles of receptor and ligand endocytosis in regulating Notch signaling. *Development*, 132, 1751-62 (2005)
99. Lai, E. C.: Notch signaling: control of cell communication and cell fate. *Development*, 131, 965-73 (2004)
100. Mukherjee, A., A. Veraksa, A. Bauer, C. Rosse, J. Camonis & S. Artavanis-Tsakonas: Regulation of Notch signalling by non-visual beta-arrestin. *Nat Cell Biol*, 7, 1191-201 (2005)
101. Frise, E., J. A. Knoblich, S. Younger-Shepherd, L. Y. Jan & Y. N. Jan: The Drosophila Numb protein inhibits signaling of the Notch receptor during cell-cell interaction in sensory organ lineage. *Proc Natl Acad Sci U S A*, 93, 11925-32 (1996)
102. Hubbard, E. J., G. Wu, J. Kitajewski & I. Greenwald: sel-10, a negative regulator of lin-12 activity in

Caenorhabditis elegans, encodes a member of the CDC4 family of proteins. *Genes Dev*, 11, 3182-93 (1997)
103. Baker, N. E.: Master regulatory genes; telling them what to do. *Bioessays*, 23, 763-6 (2001)
104. Matheny, S. A., C. Chen, R. L. Kortum, G. L. Razidlo, R. E. Lewis & M. A. White: Ras regulates assembly of mitogenic signalling complexes through the effector protein IMP. *Nature*, 427, 256-60 (2004)
105. Morrison, D. K. & R. J. Davis: Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu Rev Cell Dev Biol*, 19, 91-118 (2003)
106. Bumeister, R., C. Rosse, A. Anselmo, J. Camonis & M. A. White: CNK2 couples NGF signal propagation to multiple regulatory cascades driving cell differentiation. *Curr Biol*, 14, 439-45 (2004)
107. Breslauer, D. N., P. J. Lee & L. P. Lee: Microfluidics-based systems biology. *Mol Biosyst*, 2, 97-112 (2006)
108. Richardson, T. P., M. C. Peters, A. B. Ennett & D. J. Mooney: Polymeric system for dual growth factor delivery. *Nat Biotechnol*, 19, 1029-34 (2001)
109. Luo, Y. & M. S. Shoichet: A photolabile hydrogel for guided three-dimensional cell growth and migration. *Nat Mater*, 3, 249-53 (2004)
110. Mapili, G., Y. Lu, S. Chen & K. Roy: Laser-layered microfabrication of spatially patterned functionalized tissue-engineering scaffolds. *J Biomed Mater Res B Appl Biomater*, 75, 414-24 (2005)
111. Koh, W. G., A. Revzin & M. V. Pishko: Poly (ethylene glycol) hydrogel microstructures encapsulating living cells. *Langmuir*, 18, 2459-62 (2002)
112. Burdick, J. A., A. Khademhosseini & R. Langer: Fabrication of gradient hydrogels using a microfluidics/photopolymerization process. *Langmuir*, 20, 5153-6 (2004)
113. Dertinger, S. K., X. Jiang, Z. Li, V. N. Murthy & G. M. Whitesides: Gradients of substrate-bound laminin orient axonal specification of neurons. *Proc Natl Acad Sci U S A*, 99, 12542-7 (2002)
114. Taylor, A. M., M. Blurton-Jones, S. W. Rhee, D. H. Cribbs, C. W. Cotman & N. L. Jeon: A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nat Methods*, 2, 599-605 (2005)
115. Chung, B. G., L. A. Flanagan, S. W. Rhee, P. H. Schwartz, A. P. Lee, E. S. Monuki & N. L. Jeon: Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. *Lab Chip*, 5, 401-6 (2005)
116. Harbers, G. M. & K. E. Healy: The effect of ligand type and density on osteoblast adhesion, proliferation, and matrix mineralization. *J Biomed Mater Res A*, 75, 855-69 (2005)
117. Barber, T. A., L. J. Gamble, D. G. Castner & K. E. Healy: In vitro characterization of peptide-modified p (AAm-co-EG/AAc) IPN-coated titanium implants. *J Orthop Res*, 24, 1366-76 (2006)
118. Gerhart, J.: 1998 Warkany lecture: signaling pathways in development. *Teratology*, 60, 226-39 (1999)
119. Kamakura, S., K. Oishi, T. Yoshimatsu, M. Nakafuku, N. Masuyama & Y. Gotoh: Hes binding to STAT3 mediates crosstalk between Notch and JAK-STAT signalling. *Nat Cell Biol*, 6, 547-54 (2004)
120. Galceran, J., C. Sustmann, S. C. Hsu, S. Folberth & R. Grosschedl: LEF1-mediated regulation of Delta-like1 links Wnt and Notch signaling in somitogenesis. *Genes Dev*, 18, 2718-23 (2004)
121. Duncan, A. W., F. M. Rattis, L. N. DiMascio, K. L. Congdon, G. Pazianos, C. Zhao, K. Yoon, J. M. Cook, K. Willert, N. Gaiano & T. Reya: Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. *Nat Immunol*, 6, 314-22 (2005)
122. Nicolas, M., A. Wolfer, K. Raj, J. A. Kummer, P. Mill, M. van Noort, C. C. Hui, H. Clevers, G. P. Dotto & F. Radtke: Notch1 functions as a tumor suppressor in mouse skin. *Nat Genet*, 33, 416-21 (2003)
123. Widelitz, R. B., T. X. Jiang, C. W. Chen, N. S. Stott & C. M. Chuong: Wnt-7a in feather morphogenesis: involvement of anterior-posterior asymmetry and proximal-distal elongation demonstrated with an in vitro reconstitution model. *Development*, 126, 2577-87 (1999)
124. Blokzijl, A., C. Dahlqvist, E. Reissmann, A. Falk, A. Moliner, U. Lendahl & C. F. Ibanez: Cross-talk between the Notch and TGF-beta signaling pathways mediated by interaction of the Notch intracellular domain with Smad3. *J Cell Biol*, 163, 723-8 (2003)
125. Dahlqvist, C., A. Blokzijl, G. Chapman, A. Falk, K. Dannaeus, C. F. Ibanez & U. Lendahl: Functional Notch signaling is required for BMP4-induced inhibition of myogenic differentiation. *Development*, 130, 6089-99 (2003)
126. Itoh, F., S. Itoh, M. J. Goumans, G. Valdimarsdottir, T. Iso, G. P. Dotto, Y. Hamamori, L. Kedes, M. Kato & P. ten Dijke Pt: Synergy and antagonism between Notch and BMP receptor signaling pathways in endothelial cells. *Embo J*, 23, 541-51 (2004)
127. Beck, C. W., B. Christen & J. M. Slack: Molecular pathways needed for regeneration of spinal cord and muscle in a vertebrate. *Dev Cell*, 5, 429-39 (2003)
128. Sun, Y., W. Lowther, K. Kato, C. Bianco, N. Kenney, L. Strizzi, D. Raafat, M. Hirota, N. I. Khan, S. Bargo, B. Jones, D. Salomon & R. Callahan: Notch4 intracellular domain binding to Smad3 and inhibition of the TGF-beta signaling. *Oncogene*, 24, 5365-74 (2005)
129. Lutolf, M. P., J. L. Lauer-Fields, H. G. Schmoekel, A. T. Metters, F. E. Weber, G. B. Fields & J. A. Hubbell: Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics. *Proc Natl Acad Sci U S A*, 100, 5413-8 (2003)
130. Grande, D. A., J. Mason, E. Light & D. Dines: Stem cells as platforms for delivery of genes to enhance cartilage repair. *J Bone Joint Surg Am*, 85-A Suppl 2, 111-6 (2003)
131. Edwards, P. C., S. Ruggiero, J. Fantasia, R. Burakoff, S. M. Moorji, E. Paric, P. Razzano, D. A. Grande & J. M. Mason: Sonic hedgehog gene-enhanced tissue engineering for bone regeneration. *Gene Ther*, 12, 75-86 (2005)
132. Kellner, K., K. Lang, A. Papadimitriou, U. Leser, S. Milz, M. B. Schulz, T. Blunk & A. Gopferich: Effects of hedgehog proteins on tissue engineering of cartilage in vitro. *Tissue Eng*, 8, 561-72 (2002)
133. Yoo, A. S., C. Bais & I. Greenwald: Crosstalk between the EGFR and LIN-12/Notch pathways in C. elegans vulval development. *Science*, 303, 663-6 (2004)
134. Cooper, M. T. & S. J. Bray: R7 photoreceptor specification requires Notch activity. *Curr Biol*, 10, 1507-10 (2000)

135. Flores, G. V., H. Duan, H. Yan, R. Nagaraj, W. Fu, Y. Zou, M. Noll & U. Banerjee: Combinatorial signaling in the specification of unique cell fates. *Cell*, 103, 75-85 (2000)
136. Tomlinson, A. & G. Struhl: Delta/Notch and Boss/Sevenless signals act combinatorially to specify the Drosophila R7 photoreceptor. *Mol Cell*, 7, 487-95 (2001)
137. Tsuda, L., R. Nagaraj, S. L. Zipursky & U. Banerjee: An EGFR/Ebi/Sno pathway promotes delta expression by inactivating Su (H)/SMRTER repression during inductive notch signaling. *Cell*, 110, 625-37 (2002)
138. Chen, N. & I. Greenwald: The lateral signal for LIN-12/Notch in C. elegans vulval development comprises redundant secreted and transmembrane DSL proteins. *Dev Cell*, 6, 183-92 (2004)
139. Ikeya, T. & S. Hayashi: Interplay of Notch and FGF signaling restricts cell fate and MAPK activation in the Drosophila trachea. *Development*, 126, 4455-63 (1999)
140. Carmena, A., E. Buff, M. S. Halfon, S. Gisselbrecht, F. Jimenez, M. K. Baylies & A. M. Michelson: Reciprocal regulatory interactions between the Notch and Ras signaling pathways in the Drosophila embryonic mesoderm. *Dev Biol*, 244, 226-42 (2002)
141. Weijzen, S., P. Rizzo, M. Braid, R. Vaishnav, S. M. Jonkheer, A. Zlobin, B. A. Osborne, S. Gottipati, J. C. Aster, W. C. Hahn, M. Rudolf, K. Siziopikou, W. M. Kast & L. Miele: Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. *Nat Med*, 8, 979-86 (2002)
142. Small, D., D. Kovalenko, R. Soldi, A. Mandinova, V. Kolev, R. Trifonova, C. Bagala, D. Kacer, C. Battelli, L. Liaw, I. Prudovsky & T. Maciag: Notch activation suppresses fibroblast growth factor-dependent cellular transformation. *J Biol Chem*, 278, 16405-13 (2003)
143. Stella, M. C., L. Trusolino, S. Pennacchietti & P. M. Comoglio: Negative feedback regulation of Met-dependent invasive growth by Notch. *Mol Cell Biol*, 25, 3982-96 (2005)
144. Hasson, P., N. Egoz, C. Winkler, G. Volohonsky, S. Jia, T. Dinur, T. Volk, A. J. Courey & Z. Paroush: EGFR signaling attenuates Groucho-dependent repression to antagonize Notch transcriptional output. *Nat Genet*, 37, 101-5 (2005)
145. Holland, T. A., Y. Tabata & A. G. Mikos: Dual growth factor delivery from degradable oligo (poly (ethylene glycol) fumarate) hydrogel scaffolds for cartilage tissue engineering. *J Control Release*, 101, 111-25 (2005)
146. Simmons, C. A., E. Alsberg, S. Hsiong, W. J. Kim & D. J. Mooney: Dual growth factor delivery and controlled scaffold degradation enhance in vivo bone formation by transplanted bone marrow stromal cells. *Bone*, 35, 562-9 (2004)
147. Takayama, S., E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber & G. M. Whitesides: Subcellular positioning of small molecules. *Nature*, 411, 1016 (2001)
148. Takayama, S., J. C. McDonald, E. Ostuni, M. N. Liang, P. J. Kenis, R. F. Ismagilov & G. M. Whitesides: Patterning cells and their environments using multiple laminar fluid flows in capillary networks. *Proc Natl Acad Sci U S A*, 96, 5545-8 (1999)
149. Wagers, A. J. & I. M. Conboy: Cellular and molecular signatures of muscle regeneration: current concepts and controversies in adult myogenesis. *Cell*, 122, 659-67 (2005)
150. Conboy, I. M., M. J. Conboy, G. M. Smythe & T. A. Rando: Notch-mediated restoration of regenerative potential to aged muscle. *Science*, 302, 1575-7 (2003)
151. Nakamura, Y., S. Sakakibara, T. Miyata, M. Ogawa, T. Shimazaki, S. Weiss, R. Kageyama & H. Okano: The bHLH gene hes1 as a repressor of the neuronal commitment of CNS stem cells. *J Neurosci*, 20, 283-93 (2000)
152. Das, A. V., K. B. Mallya, X. Zhao, F. Ahmad, S. Bhattacharya, W. B. Thoreson, G. V. Hegde & I. Ahmad: Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling. *Dev Biol*, 299, 283-302 (2006)
153. Raymond, P. A., L. K. Barthel, R. L. Bernardos & J. J. Perkowski: Molecular characterization of retinal stem cells and their niches in adult zebrafish. *BMC Dev Biol*, 6, 36 (2006)
154. Bernardos, R. L., S. I. Lentz, M. S. Wolfe & P. A. Raymond: Notch-Delta signaling is required for spatial patterning and Muller glia differentiation in the zebrafish retina. *Dev Biol*, 278, 381-95 (2005)
155. Dang, L., K. Yoon, M. Wang & N. Gaiano: Notch3 signaling promotes radial glial/progenitor character in the mammalian telencephalon. *Dev Neurosci*, 28, 58-69 (2006)
156. Kohyama, J., A. Tokunaga, Y. Fujita, H. Miyoshi, T. Nagai, A. Miyawaki, K. Nakao, Y. Matsuzaki & H. Okano: Visualization of spatiotemporal activation of Notch signaling: live monitoring and significance in neural development. *Dev Biol*, 286, 311-25 (2005)
157. Grandbarbe, L., J. Bouissac, M. Rand, M. Hrabe de Angelis, S. Artavanis-Tsakonas & E. Mohier: Delta-Notch signaling controls the generation of neurons/glia from neural stem cells in a stepwise process. *Development*, 130, 1391-402 (2003)
158. Ge, W., K. Martinowich, X. Wu, F. He, A. Miyamoto, G. Fan, G. Weinmaster & Y. E. Sun: Notch signaling promotes astrogliogenesis via direct CSL-mediated glial gene activation. *J Neurosci Res*, 69, 848-60 (2002)
159. Tanigaki, K., F. Nogaki, J. Takahashi, K. Tashiro, H. Kurooka & T. Honjo: Notch1 and Notch3 instructively restrict bFGF-responsive multipotent neural progenitor cells to an astroglial fate. *Neuron*, 29, 45-55 (2001)
160. Hitoshi, S., T. Alexson, V. Tropepe, D. Donoviel, A. J. Elia, J. S. Nye, R. A. Conlon, T. W. Mak, A. Bernstein & D. van der Kooy: Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. *Genes Dev*, 16, 846-58 (2002)
161. Bjerknes, M. & H. Cheng: Clonal analysis of mouse intestinal epithelial progenitors. *Gastroenterology*, 116, 7-14 (1999)
162. Crosnier, C., D. Stamataki & J. Lewis: Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet*, 7, 349-59 (2006)
163. Schroder, N. & A. Gossler: Expression of Notch pathway components in fetal and adult mouse small intestine. *Gene Expr Patterns*, 2, 247-50 (2002)
164. Chadwick, N., M. C. Nostro, M. Baron, R. Mottram, G. Brady & A. M. Buckle: Notch Signaling Induces

Apoptosis in Primary Human CD34+ Hematopoietic Progenitor Cells. *Stem Cells*, 25, 203-10 (2007)
165. Burns, C. E., D. Traver, E. Mayhall, J. L. Shepard & L. I. Zon: Hematopoietic stem cell fate is established by the Notch-Runx pathway. *Genes Dev*, 19, 2331-42 (2005)
166. Kumano, K., S. Chiba, A. Kunisato, M. Sata, T. Saito, E. Nakagami-Yamaguchi, T. Yamaguchi, S. Masuda, K. Shimizu, T. Takahashi, S. Ogawa, Y. Hamada & H. Hirai: Notch1 but not Notch2 is essential for generating hematopoietic stem cells from endothelial cells. *Immunity*, 18, 699-711 (2003)
167. Tsukumo, S., K. Hirose, Y. Maekawa, K. Kishihara & K. Yasutomo: Lunatic fringe controls T cell differentiation through modulating notch signaling. *J Immunol*, 177, 8365-71 (2006)
168. Garcia-Peydro, M., V. G. de Yebenes & M. L. Toribio: Notch1 and IL-7 receptor interplay maintains proliferation of human thymic progenitors while suppressing non-T cell fates. *J Immunol*, 177, 3711-20 (2006)
169. Guidos, C. J.: Synergy between the pre-T cell receptor and Notch: cementing the alphabeta lineage choice. *J Exp Med*, 203, 2233-7 (2006)
170. Ellisen, L. W., J. Bird, D. C. West, A. L. Soreng, T. C. Reynolds, S. D. Smith & J. Sklar: TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell*, 66, 649-61 (1991)
171. Allman, D., F. G. Karnell, J. A. Punt, S. Bakkour, L. Xu, P. Myung, G. A. Koretzky, J. C. Pui, J. C. Aster & W. S. Pear: Separation of Notch1 promoted lineage commitment and expansion/transformation in developing T cells. *J Exp Med*, 194, 99-106 (2001)
172. Weng, A. P., A. A. Ferrando, W. Lee, J. P. t. Morris, L. B. Silverman, C. Sanchez-Irizarry, S. C. Blacklow, A. T. Look & J. C. Aster: Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science*, 306, 269-71 (2004)
173. Maillard, I., T. Fang & W. S. Pear: Regulation of lymphoid development, differentiation, and function by the Notch pathway. *Annu Rev Immunol*, 23, 945-74 (2005)
Spinner, N. B., R. P. Colliton, C. Crosnier, I. D. Krantz, M. Hadchouel & M. Meunier-Rotival: Jagged1 mutations in alagille syndrome. *Hum Mutat*, 17, 18-33 (2001)
174. Li, L., I. D. Krantz, Y. Deng, A. Genin, A. B. Banta, C. C. Collins, M. Qi, B. J. Trask, W. L. Kuo, J. Cochran, T. Costa, M. E. Pierpont, E. B. Rand, D. A. Piccoli, L. Hood & N. B. Spinner: Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat Genet*, 16, 243-51 (1997)
175. Oda, T., A. G. Elkahloun, B. L. Pike, K. Okajima, I. D. Krantz, A. Genin, D. A. Piccoli, P. S. Meltzer, N. B. Spinner, F. S. Collins & S. C. Chandrasekharappa: Mutations in the human Jagged1 gene are responsible for Alagille syndrome. *Nat Genet*, 16, 235-42 (1997)
176. Morrissette, J. D., R. P. Colliton & N. B. Spinner: Defective intracellular transport and processing of JAG1 missense mutations in Alagille syndrome. *Hum Mol Genet*, 10, 405-13 (2001)
177. Krantz, I. D., D. A. Piccoli & N. B. Spinner: Alagille syndrome. *J Med Genet*, 34, 152-7 (1997)
178. Krantz, I. D.: Alagille syndrome: chipping away at the tip of the iceberg. *Am J Med Genet*, 112, 160-2 (2002)
179. Emerick, K. M., E. B. Rand, E. Goldmuntz, I. D. Krantz, N. B. Spinner & D. A. Piccoli: Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. *Hepatology*, 29, 822-9 (1999)
180. Gessler, M., K. P. Knobeloch, A. Helisch, K. Amann, N. Schumacher, E. Rohde, A. Fischer & C. Leimeister: Mouse gridlock: no aortic coarctation or deficiency, but fatal cardiac defects in Hey2 -/- mice. *Curr Biol*, 12, 1601-4 (2002)
181. Donovan, J., A. Kordylewska, Y. N. Jan & M. F. Utset: Tetralogy of fallot and other congenital heart defects in Hey2 mutant mice. *Curr Biol*, 12, 1605-10 (2002)
182. Mortier, G. R., R. S. Lachman, M. Bocian & D. L. Rimoin: Multiple vertebral segmentation defects: analysis of 26 new patients and review of the literature. *Am J Med Genet*, 61, 310-9 (1996)
183. Bulman, M. P., K. Kusumi, T. M. Frayling, C. McKeown, C. Garrett, E. S. Lander, R. Krumlauf, A. T. Hattersley, S. Ellard & P. D. Turnpenny: Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. *Nat Genet*, 24, 438-41 (2000)
184. Kusumi, K., E. S. Sun, A. W. Kerrebrock, R. T. Bronson, D. C. Chi, M. S. Bulotsky, J. B. Spencer, B. W. Birren, W. N. Frankel & E. S. Lander: The mouse pudgy mutation disrupts Delta homologue Dll3 and initiation of early somite boundaries. *Nat Genet*, 19, 274-8 (1998)
185. Dunwoodie, S. L., M. Clements, D. B. Sparrow, X. Sa, R. A. Conlon & R. S. Beddington: Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene Dll3 are associated with disruption of the segmentation clock within the presomitic mesoderm. *Development*, 129, 1795-806 (2002)
186. Zhang, N., C. R. Norton & T. Gridley: Segmentation defects of Notch pathway mutants and absence of a synergistic phenotype in lunatic fringe/radical fringe double mutant mice. *Genesis*, 33, 21-8 (2002)
187. Zhang, N. & T. Gridley: Defects in somite formation in lunatic fringe-deficient mice. *Nature*, 394, 374-7 (1998)
188. Joutel, A., C. Corpechot, A. Ducros, K. Vahedi, H. Chabriat, P. Mouton, S. Alamowitch, V. Domenga, M. Cecillion, E. Marechal, J. Maciazek, C. Vayssiere, C. Cruaud, E. A. Cabanis, M. M. Ruchoux, J. Weissenbach, J. F. Bach, M. G. Bousser & E. Tournier-Lasserve: Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*, 383, 707-10 (1996)
189. Haritunians, T., J. Boulter, C. Hicks, J. Buhrman, G. DiSibio, C. Shawber, G. Weinmaster, D. Nofziger & C. Schanen: CADASIL Notch3 mutant proteins localize to the cell surface and bind ligand. *Circ Res*, 90, 506-8 (2002)
190. Joutel, A., F. Andreux, S. Gaulis, V. Domenga, M. Cecillon, N. Battail, N. Piga, F. Chapon, C. Godfrain & E. Tournier-Lasserve: The ectodomain of the Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients. *J Clin Invest*, 105, 597-605 (2000)
191. Ruchoux, M. M., V. Domenga, P. Brulin, J. Maciazek, S. Limol, E. Tournier-Lasserve & A. Joutel: Transgenic mice expressing mutant Notch3 develop vascular alterations characteristic of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Am J Pathol*, 162, 329-42 (2003)

192. Hubbell, J. A.: Bioactive biomaterials. *Curr Opin Biotechnol*, 10, 123-9 (1999)
193. Griffith, L. G.: Emerging design principles in biomaterials and scaffolds for tissue engineering. *Ann N Y Acad Sci*, 961, 83-95 (2002)
194. Zisch, A. H., U. Schenk, J. C. Schense, S. E. Sakiyama-Elbert & J. A. Hubbell: Covalently conjugated VEGF--fibrin matrices for endothelialization. *J Control Release*, 72, 101-13 (2001)
195. Sakiyama-Elbert, S. E., A. Panitch & J. A. Hubbell: Development of growth factor fusion proteins for cell-triggered drug delivery. *Faseb J*, 15, 1300-2 (2001)
196. Halstenberg, S., A. Panitch, S. Rizzi, H. Hall & J. A. Hubbell: Biologically engineered protein-graft-poly (ethylene glycol) hydrogels: a cell adhesive and plasmin-degradable biosynthetic material for tissue repair. *Biomacromolecules*, 3, 710-23 (2002)
197. Pratt, A. B., F. E. Weber, H. G. Schmoekel, R. Muller & J. A. Hubbell: Synthetic extracellular matrices for in situ tissue engineering. *Biotechnol Bioeng*, 86, 27-36 (2004)
198. Kim, S. & K. E. Healy: Synthesis and characterization of injectable poly (N-isopropylacrylamide-co-acrylic acid) hydrogels with proteolytically degradable cross-links. *Biomacromolecules*, 4, 1214-23 (2003)
199. Gobin, A. S. & J. L. West: Cell migration through defined, synthetic ECM analogs. *Faseb J*, 16, 751-3 (2002)
200. Peppas, N. A., Y. Huang, M. Torres-Lugo, J. H. Ward & J. Zhang: Physicochemical foundations and structural design of hydrogels in medicine and biology. *Annu Rev Biomed Eng*, 2, 9-29 (2000)
201. Hoffman, A. S.: Hydrogels for biomedical applications. *Adv Drug Deliv Rev*, 54, 3-12 (2002)
202. Drury, J. L. & D. J. Mooney: Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials*, 24, 4337-51 (2003)
203. Zisch, A. H., M. P. Lutolf, M. Ehrbar, G. P. Raeber, S. C. Rizzi, N. Davies, H. Schmokel, D. Bezuidenhout, V. Djonov, P. Zilla & J. A. Hubbell: Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularized tissue growth. *Faseb J*, 17, 2260-2 (2003)
204. Ehrbar, M., V. G. Djonov, C. Schnell, S. A. Tschanz, G. Martiny-Baron, U. Schenk, J. Wood, P. H. Burri, J. A. Hubbell & A. H. Zisch: Cell-demanded liberation of VEGF121 from fibrin implants induces local and controlled blood vessel growth. *Circ Res*, 94, 1124-32 (2004)
205. Juan-Pardo, E. M., M. Hoang, & I. M. Conboy: Geometric control of myogenic cell fate. *Int J Nanomedicine*, 1, 203-212 (2006)

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