

**PW 35:**  
**Cardiac, smooth and  
other muscles**

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| PW35-434   | <p><b><u>HUMAN SERCA2A CONTROLS VASCULAR SMOOTH MUSCLE CELL PROLIFERATION VIA INHIBITION OF BASAL CA<sup>2+</sup> INFLUX AND NFAT.</u></b><br/> LIPSKAIA L<sup>1</sup>, COLOMBE A<sup>2</sup>, LEHOUX S<sup>3</sup>, ESPOSITO B<sup>3</sup>, ATASSI F<sup>2</sup>, LE PRINCE P<sup>4</sup>, BONNET N<sup>4</sup>, LE FEUVRE C<sup>4</sup>, HATEM S<sup>2</sup>, HAJJAR R<sup>1</sup>, LOMPRES AM<sup>2</sup><br/> (1) MSSM, New York, USA. (2) INSERM U621, Paris, FRANCE. (3) INSERM 689, Paris, FRANCE. (4) Hôpital Pitié-Salpêtrière, Paris, FRANCE.</p>   |
| To contact the author::<br>lipskaia@chups.jussieu.fr | <p>Coronary restenosis, results mainly from the proliferation of vascular smooth muscle cells (VSMC). Here, we determine whether in human arteries, the cardiac isoform of the sarco/endoplasmic reticulum calcium ATPase, SERCA2a controls VSMC proliferation via calcium-dependent calcineurin/NFAT signalling pathway. In atherosclerotic coronary arteries (CA) SERCA2a and the ryanodine receptor (RyR2) were expressed only in differentiated VSMC from the media but not in the neointima, containing mainly dedifferentiated cells. Primary CA VSMCs lost SERCA2a and RyR within 3 days after induction of proliferation. SERCA2a gene transfer inhibited VSMC proliferation through inhibition of NFAT leading to down regulation of cell cycle controlling proteins cyclin D1 and Erg1. Single-channel patch-clamp recording showed that SERCA2a gene transfer inhibited basal voltage-independent Ca<sup>2+</sup> influx. Promoter-reporter assay showed that in human VSMCs NFAT was activated by store-dependent Ca<sup>2+</sup> influx, inhibited by depolarisation-induced Ca<sup>2+</sup> influx and its transcriptional activity strongly correlated with proliferative state, as demonstrated by Ca<sup>2+</sup> channels blockers, expression of VIVIT (NFAT inhibitory peptide) or constitutively active NFAT. Furthermore, SERCA2a gene transfer prevented vascular remodelling and reduced neointima formation in an <i>ex-vivo</i> model of injury: the left internal mammary arteries (IMA), injured and kept 2 weeks in organ culture under constant pressure and flow (intima/media ratio was 0.07 ± 0.01 vs 0.40 ± 0.03 in <math>\square</math>Gal-infected arteries, p&lt;0.001, n=5). In conclusion, in human SERCA2a controls proliferation of VSMC and neointima formation <i>via</i> inhibition of basal voltage-independent Ca<sup>2+</sup> influx and transcription factor NFAT. These findings could have potential implications for treatment of intervention-induced restenosis.</p> |

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| PW35-435   | <p><b><u>MOLECULAR CONTROLS OF VISCERAL SMOOTH MUSCLE CELL DIFFERENTIATION IN VERTEBRATE EMBRYOS</u></b><br/> NOTARNICOLA C<sup>1</sup>, LE GUEN L<sup>1</sup>, DE SANTA BARBARA P<sup>1</sup><br/> (1) INSERM ERI 25, Muscle and Pathologies, Montpellier, FRANCE.</p>  |
| <p>To contact the author::<br/> notarnicola@montp.inserm.fr.</p> | <p>Despite significant advances in the description of molecular controls of gut development in different animal models, little works have been done on the pathways involved during visceral smooth muscle cell differentiation. This differentiation is often affected in patients with congenital gut malformations. These malformations account for a significant percentage of all congenital defects, and the molecular description of gut development would be beneficial in diagnostic and therapeutic treatment of these common human gastrointestinal disorders.</p> <p>Our group focuses on the identification and the functional study of the molecular mechanisms that control the differentiation of the visceral smooth muscle cells (SMCs). We use the chick embryo as animal model, and techniques from development (misexpression of cDNAs by avian retroviral infection in the mesodermal layer). With a microarray approach, we found only 55 genes regulated during the differentiation of SMCs and let us hypothesize that we identified important molecular players that could be essential to trigger the differentiation of the mesenchymal cells into SMCs. The limited number of genes gave us the opportunities to study the expression patterns of different candidates. We already analyzed the status of the FGF pathways activation and found that the FGF signaling pathways is activated in the mesodermal cells, but this pathway is specifically down-regulated during the differentiation of the visceral SMCs. In addition, when we maintained the activation of the FGF pathways, we were able to avoid the visceral SMCs differentiation. These experiments showed that the control and timing of FGF pathway activation is essential for trigger of SMCs differentiation. New potential smooth muscle markers have been isolated such as <i>Hermes</i>, a RNA-binding protein containing an RNA-recognition motif. We observed expression of <i>Hermes</i> in the differentiated SMCs in the digestive tract close to <math>\square</math> SMA expression. Functional studies of these factors are currently investigated in our laboratory.</p> |

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| PW35-436  | <p><b><u>INTERMUSCULAR TENDONS ARE ESSENTIAL FOR THE DEVELOPMENT OF VERTEBRATE STOMACH</u></b><br/> LE GUEN L<sup>1</sup>, NOTARNICOLA C<sup>1</sup>, DE SANTA BARBARA P<sup>1</sup><br/> (1) INSERM ERI 25, Muscle and Pathologies, Montpellier, FRANCE.</p>   |
| To contact the author:<br>desanta@montp.inserm.fr | <p>The gastrointestinal tract is a vital organ system present in all multicellular animals initially derived from a simple tubal structure. The morphology of the gut requires reciprocal signaling between the mesoderm and endoderm during development. In order to identify and functionally study the mechanisms that control the specific development of the stomach, we performed a microarray study to obtain the gene expression profiles in the stomach, and in the colon. Analyses of these results confirmed some genes (<i>BapX1</i>, <i>Hoxa5</i>) and gave us new candidate genes (<i>Scleraxis</i>, <i>Tenomodulin</i>).</p> <p><i>Scleraxis</i>, a member of the basic-helix-loop-helix family has been reported as a marker of tendons. We identified cells expressing <i>Scleraxis</i> in the stomach as tendon cells adjacent to visceral smooth muscle cells. We found that <i>Scleraxis</i> expression is present in mouse stomach, with expression limited to small domains, highest adaptation of the stomach between species. In order to investigate more directly the role of <i>Scleraxis</i> during the development of the stomach, we used the avian retroviral system to specifically misexpress or inactivated <i>Scleraxis</i> in the stomach mesoderm. Using specific <i>Scleraxis</i> siRNA cloned into avian retroviruses, we observed that thin malformed stomachs are associated with a strong diminution of <i>Scleraxis</i> expression suggesting that <i>Scleraxis</i> expression is essential for the correct development of the stomach. Ectopic expression of <i>Scleraxis</i> into the whole stomach mesoderm leads to drastic morphological change characterized by a thick stomach associated with a strong downregulation of smooth muscle markers but no induction of ectopic tendinous structure. These experiments showed that <i>Scleraxis</i> is able to inhibit the differentiation of the smooth muscle cells and more surprising to inhibit the differentiation of the tendon.</p> <p>In summary, we identified the presence of intermuscular tendons in vertebrate stomach, and showed that the function of <i>Scleraxis</i> could be to pattern the tendon domains into avian stomach.</p> |

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| PW35-437  | <p><b><u>INVESTIGATING THE FUNCTION OF TBX1 IN CRANIOFACIAL MUSCLE DEVELOPMENT</u></b></p> <p>GRIFONE R<sup>1</sup>, JARRY T<sup>1</sup>, KELLY RG<sup>1</sup><br/> (1) Developmental Biology Institute of Marseilles - Luminy, Marseilles, FRANCE.</p>   |
| To contact the author::<br>grifone@ibdml.univ-mrs.fr. | <p>Vertebrate craniofacial and trunk myogenesis are regulated by distinct genetic programs. Branchiomeric craniofacial muscles regulate jaw opening and closing, facial expression and laryngeal and pharyngeal function. These muscles correspond to the gill musculature of fish and originate not from the somites, source of trunk and limb muscles, but from pharyngeal mesoderm. Prior to skeletal muscle specification branchiomeric muscle progenitor cells share the genetic program of a population of adjacent myocardial progenitor cells termed the second heart field which give rise to the right ventricle and outflow tract of the heart (Grifone and Kelly, 2007, Trends Genet 23, 365-9). <i>Tbx1</i>, murine homologue of the major DiGeorge/del22q11.2 syndrome candidate gene <i>TBX1</i>, is required for robust specification of branchiomeric craniofacial muscles in pharyngeal mesoderm (Kelly et al, 2004, Hum Mol Genet 13, 289-40). Here we examine the properties of branchiomeric and somite-derived muscle development in <i>Tbx1</i> mutant embryos. Using immunohistochemistry we demonstrate that the myogenic regulatory factors Myf5 and MyoD are first activated in <i>Tbx1</i> and <i>Isl1</i>-positive cells within core arch mesoderm. Despite altered fiber-type distributions and pharyngeal muscle weakness in <i>TBX1</i> haploinsufficient del22q11.2 syndrome patients, myosin heavy chain type II and type I fiber distributions are indistinguishable in <i>Tbx1</i><sup>+/+</sup> and <i>Tbx1</i><sup>+/-</sup> adult masseter and pharyngeal constrictor muscles. Furthermore, type I fibers are distributed normally in hypoplastic branchiomeric muscles which form sporadically in <i>Tbx1</i><sup>-/-</sup> embryos. These sporadic muscles contain Pax7 positive cells and primary myocytes isolated from <i>Tbx1</i><sup>-/-</sup> muscles have indistinguishable proliferative and myogenic differentiation properties from wild-type muscles in culture. A subset of somite-derived muscles express <i>Tbx1</i>, including certain limb muscles. These muscles display normal patterning, growth, fiber-type distribution and maturation in the absence of <i>Tbx1</i>. Together, our results suggest that the major role of <i>Tbx1</i> during skeletal muscle development is restricted to myogenic specification of branchiomeric muscles.</p> |

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| PW35-438 | <p><b><u>OCT-4 A PRIMARY EVENT IN SOX17-MEDIATED SPECIFICATION OF CARDIAC MESODERM</u></b><br/>         STEFANOVIC S<sup>1</sup>, ABBOUD N<sup>1</sup>, DESILETS S<sup>1</sup>, PUCEAT M<sup>1</sup><br/>         (1) INSERM/Evry University UMR861, I-STEM/AFM, Evry, FRANCE.</p>  |
|          | <p>The embryonic stem cell (ESC) specific protein Oct-4, is one of the earliest transcription factor in the embryo. Together with Sox2 and Nanog, Oct-4 cooperatively maintains the pluripotency of ESC through a tightly regulated transcriptional loop. However, a new function of this transcription factor has recently emerged. There are now valuable insights regarding the role of Oct-4 in the process of cell differentiation. We recently showed that upregulation of Oct-4 drives Mouse ESC toward a cardiogenic fate. We now report that this phenomenon is conserved in Human ESC. Furthermore, we uncovered the transcriptional mechanisms underlying this event. We found that the primary event triggered by Oct-4 upregulation is the induction of Sox17, an endodermal marker recently reported as a key factor in the formation of cardiac mesoderm. Using a ChIP assay in both Mouse and Human ESC, we found that an increase in Oct-4 level of expression displaces its interaction with Sox2 to target Sox17 DNA elements. This results in a transcriptional activation of Sox17 as monitored by real time PCR and cell immunofluorescence. Then induced endodermal cells act through a paracrine effect. These cells secrete cardiogenic factors in the medium surrounding colonies of ESC. This results in a specification of both Mouse and Human ESC toward a cardiac lineage. We thus delineated an Oct-4 mediated cardiogenic transcriptional network, conserved in both Mouse and Human ESC. These findings should advance our understanding of biological processes underlying early cardiogenesis and in turn better comprehend origins of genetic congenital diseases.</p> |
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| PW35-439                                       | <p><b><u>ECTOPIC FETAL CARDIAC GRAFT : A CONVENIENT EXPERIMENTAL MODEL OF HEART RECONSTRUCTION</u></b><br/> DELRÉE P<sup>1</sup>, COULIC V<sup>2</sup>, COLLETTE J<sup>3</sup>, GOTHOT A<sup>3</sup><br/> (1) IRSPG, Gosselies, BELGIUM. (2) CHU Brugmann (ULB), Brussels, BELGIUM.<br/> (3) Université de Liège (Ulg), Liège, BELGIUM.</p>   |
| To contact the author::<br>paul.delree@ipg.be. | <p>PURPOSE :in order to easily study heart development or reconstruction, we have developed a model of ectopic (in time and location) foetal heart graft.</p> <p>RESULTS : In this model ,rat foetal heart (E15-E20) , is grafted in the pavilion ear of an adult rat. Location in the pavilion ear protects the graft from automutilation, and allows easy studies of the development and function of the graft, during the whole course of his development, using external recording technologies. Histological studies demonstrate an early stage of extensive ischemic necrosis, followed some days after,by a reconstruction process , grossly recapitulating heart ontogenesis. This often leads to a partially functional heart with blood filled cavities, pace-maker and beating activities and to some extent with blood flow, as assessed by histological, ultrasound, IRM and electrical activity studies. Immunohistochemistry and microelectronic studies demonstrate progressive acquisition of adult myocyte phenotype of the graft cells. Furthermore the fact of grafting induces a elevation of serum IGF1 level.(50%).</p> <p>DISCUSSION : this experimental model allows study of the cellular and cytokine environment associated with cardiac reconstruction of a foetal heart .That knowledge could potentially give insights to optimize adult cardiac repair using cellular therapy. By example, modifying cytokine network during a cellular therapy of genetic diseased heart, in a way mimicking the one observed during the foetal heart graft,, could potentially favour engineered cells to engraft successfully in the diseased heart. In that context of cellular therapy, we intend to study the potential mobilisation of host stem cell induced by foetal heart grafting as well as their potential engraftment in the foetal graft.</p> |

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| PW35-440  | <p><b>BIOCOMPATIBLE NANOFILMS DRIVE DIFFERENTIATION OF STEM CELLS TOWARD A CARIOGENIC FATE</b><br/> LOUIS-TISSERAND M<sup>1</sup>, BLIN G<sup>1</sup>, PICART C<sup>2</sup>, PUCÉAT M<sup>1</sup><br/> (1) INSERM/UEVE UMR 861, I-Stem, AFM, Evry, FRANCE. (2) INSERM CNRS-UMR 5235, Montpellier, FRANCE.</p>  |
| To contact the author::<br>gblin@istem.genethon.fr. | <p>Cell therapy has emerged as a promising therapeutic option for heart failure. To such an aim, optimisation of cell engraftment is mandatory. Both cell survival and secure differentiation are likely to require an extracellular scaffold. Using nanotechnology, we engineered biocompatible polypeptide and polysaccharide multilayer films (PEMS). These included Poly (L-lysine) hydrobromide (PLL) and Hyaluronan (HA). Crosslinking of films using different amounts of dimethylaminopropyl carbodiimide (EDC) defines their stiffness.</p> <p>We found that the most plastic cells (i.e, embryonic stem cells) respond to the force exerted by their attachment to the film. This force is translated into induction of a mesodermal cardiogenic genetic program. Indeed, stem cells cultured on PEMS (PLL/HA) with increasing stiffness turned on expression of mesodermal genes. This was associated with changes in cell shape. Real time RT-PCR revealed that the most rigid films (EDC:100), mimicking the infarction scar, induced a 4, 7, 6, 4.5 fold increase in expression of Brachyury, Myocardin, Tbx6 and Mesp1,2, respectively, in comparison with the non cross-linked ones. In fact, more the films were cross-linked and in turn stiff, more the cells adhered, changed their shape and expressed mesodermal cardiogenic genes. Expression of the protein Brachyury was also observed in cells grown on rigid films (from EDC 40). Furthermore, the proliferation rate of cells also increased with the level of stiffness. Our study shows that the stiffness of films is crucial for the differentiation of stem cells towards mesodermal cardiac lineage.</p> <p>By exerting traction forces on a substrate, stem cells sense the stiffness and show dissimilar morphology and adhesive properties translated in gene transcription. Biocompatible nanofilms might represent in the future a mean to secure a cell product in therapy of heart failure.</p> |

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| PW35-441  | <p><b><u>ECTOPIC FETAL INTESTINE GRAFT AS A MODEL FOR CELLULAR THERAPY IN HIRSCHPRUNG DISEASE</u></b><br/> COULIC V<sup>1</sup>, DELRÉE P<sup>2</sup>, COLLETTE J<sup>3</sup>, GOTHOT A<sup>3</sup><br/> (1) CHU Brugmann (ULB), Brussels, BELGIUM. (2) IRSPG, Gosselies, BELGIUM.<br/> (3) University of Liege (Ulg), Liege, BELGIUM.</p>  |
| <p>To contact the author::<br/> paul.delree@ipg.be.</p> | <p><b>Hirschprung disease may be considered as an intestinal neuropathy with muscular dysfunction. Some hope for its treatment has appeared with cellular therapy.</b></p> <p><b>Aim: to test the possibility of intestinal nervous ganglia reconstruction .</b></p> <p><b>Material, methods: To study intestinal reconstruction features, syngenic fetal intestine implantation was provided in 30 Fischer rats (site: subcutaneous pouch of the ear pavilion). HE , connexin-43, nestin, vimentin and actin staining allowed evaluation of graft maturation state. Host cell participation in graft development, was evaluated by grafting fetal intestine of “wild type” mice C57BI (WT) to 30 green fluorescent protein (b-actin-GFP) transgenic mice (same site). HE , GFP and SOX-10 staining were used to identify host cells in the graft and to detect neuronal precursors.</b></p> <p><b>Results.</b> After initial graft ischaemic destruction, reconstruction and growth of the organ were observed. The reconstruction beginning corresponded to an increase of IGF-1 levels in the recipient serum, and of nestin and connexin-43 expression in the graft. Actin and vimentin were first absent and reappeared as smooth muscle differentiation started. After 1 month, muscular layers and myenteric plexuses were well-developed. In mice, GFP positive cells, coming only from the GFP host indeed, were identified in the implant, including muscular layers and many neurons.</p> <p><b>Conclusion.</b> Remaining alive initially grafted cells, as well as host cell, incorporated in the graft, grew and differentiated into the different layers of intestine, including neuronal ganglia, according to ontogenic patterns. . Moreover the graft modifies host metabolism, as increased host serum IGF-1 level testimonies. . These observations opens potentialities for cellular therapy in Hirschprung disease</p> |

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| PW35-442   | <p><b><u>RAE-1 IS A NEW MARKER FOR NEURAL STEM CELLS INVOLVED IN CELL PROLIFERATION</u></b></p> <p>CEDILE O<sup>1</sup>, POPA N<sup>1</sup>, POLLET-VILLARD X<sup>1</sup>, MONTI G<sup>2</sup>, BAGNIS C<sup>3</sup>, DURBEC P<sup>2</sup>, BOUCRAUT J<sup>1</sup></p> <p>(1) CRN2M Centre de Recherche en Neurobiologie; CNRS UMR6231, Marseille, FRANCE. (2) IBDML Institut de Biologie du Developpement de Marseille Luminy; CNRS UMR6216, Marseille, FRANCE. (3) EFS Etablissement Français du Sang Alpes Méditerranée; laboratoire de thérapie génique et cellulaire, Marseille, FRANCE.</p>   |
| To contact the author::<br>oriane.cedile@univmed.fr. | <p>More and more experiments show the role of immune cells and immunological molecules in the physiology of the nervous system including neurogenesis. A better knowledge of interactions between immune cells and neural stem cells is important for improving and controlling the capacity of either endogenous or grafted adult neural stem cells to repair the nervous system.</p> <p>We address the question of the role of Major Histocompatibility Complex-I (MHC-I) molecules in neurogenesis in the mouse model. Our main results concern the expression and functions of Rae-1, a molecule related to the MHC-I family. Rae-1 was expressed by neural stem cells and was down regulated after their differentiation. Rae-1 transcripts were known to be present only in early embryonic stages particularly in the nervous tissue. Moreover, we showed that Rae-1 was also expressed in the regions of adult neurogenesis and its expression was induced in the olfactory bulbs after olfactory axotomy.</p> <p>Rae-1 is recognized by NKG2D, the main activator receptor of the NK, NKT and T <math>\gamma\delta</math> lymphocytes. In the model of olfactory axotomy, we demonstrated a recruitment of lymphocytes in the olfactory bulbs which correlated with an increase in NKG2D transcripts. However, no NKG2D expression was detected in the subventricular zone in physiological or pathological condition. We thus questioned a role of Rae-1 in the absence of immune cells. Using a transduction approach with lentivirus coding for shRNAs targeting Rae-1, we demonstrated that Rae-1 is involved in the proliferation of neural stem cells.</p> <p>Deciphering the roles <i>in vivo</i> of Rae-1 in neurogenesis is now our main objective.</p> |

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| PW35-443  | <p><b><u>EGR1 AND EGR2 CONTROL VERTEBRATE TENDON CELL DIFFERENTIATION BY REGULATING SCLERAXIS AND COLLAGEN EXPRESSION</u></b><br/>         BLAIS F<sup>1</sup>, LÉJARD V<sup>1</sup>, MARO GS<sup>2</sup>, GILARDI-HEBENSTREIT P<sup>2</sup>, ROSSERT J<sup>3</sup>, DUPREZ D<sup>1</sup><br/>         (1) CNRS UMR 7622, Université Pierre et Marie Curie, PARIS, FRANCE. (2) INSERM U784, Ecole Normale Supérieure, PARIS, FRANCE. (3) Equipe 3 Centre de recherche des Cordeliers, Université Paris-Descartes, PARIS, FRANCE.</p>   |
| <p>To contact the author::<br/>         veronique.lejard@snv.jussieu.</p> | <p>The molecular mechanisms underlying tendon formation during vertebrate embryogenesis are still largely unknown. In <i>Drosophila</i>, tendon differentiation relies upon the transcription factor <i>stripe</i>, an Early growth response (Egr)-like transcription factor. Based on the requirement for <i>stripe</i> in tendon formation in <i>Drosophila</i>, we investigated the involvement of the homologous <i>Egr</i> family members, <i>Egr1</i> and <i>Egr2</i> in vertebrate tendon formation. We established that <i>Egr1</i> and <i>Egr2</i> transcripts are located in forming and differentiating limb tendons during chick and mouse embryogenesis. The secreted factor fibroblast growth factor-4 activated the expression of both <i>Egr</i> genes within 4 hours before the activation of the differentiation tendon markers <i>Scleraxis</i>, <i>EphA4</i> and the <i>collagens I, V</i> and <i>XII</i>. Misexpression experiments using the chick model allowed us to establish that <i>Egr</i> genes are sufficient for the expression of <i>Scleraxis</i> and tendon-associated <i>collagens</i>. Analysis of embryonic limbs from mouse mutants for <i>Egr1</i> or <i>Egr2</i> did not exhibit major modifications in tendon formation, based on the expression of <i>Scleraxis</i> and <i>collagen I</i>, suggesting redundancy between both genes in tendon formation. Finally, we showed that <i>Egr2</i> enhanced the activity of the <i>col1a1</i> tendon promoter, indicating that <i>Egr2</i> is part of the DNA-binding protein network involved in the regulation the <i>col1a1</i> expression in tendons. The endogenous tendon expression of the <i>Egrs</i>, their regulation by <i>Fgf4</i>, their sufficiency to induce tendon gene expression and their ability to bind the tendon promoter of <i>col1a1</i> all indicate that <i>Egr</i> genes control tendon cell differentiation by regulating <i>Scleraxis</i> and <i>collagen</i> expression. This represents a rare example of conserved regulatory function between homologous genes in invertebrates and vertebrates.</p> |

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| PW35-444  | <p><b><u>TSHZ3 DEFICIENCY CAUSES FUNCTIONAL RENAL TRACT OBSTRUCTION BY IMPEDING URETERIC SMOOTH MUSCLE DIFFERENTIATION</u></b></p> <p>MARTIN E<sup>1</sup>, GANNON C<sup>2</sup>, CAUBIT X<sup>1</sup>, CORE N<sup>1</sup>, FILLIPI P<sup>1</sup>, VOLA C<sup>1</sup>, GARRATT AN<sup>3</sup>, WOOLF A<sup>2</sup>, FASANO L<sup>1</sup></p> <p>(1) IBDML - Development Biology Institute of Marseille Luminy, Marseille, FRANCE.<br/> (2) Nephro-Urology Unit, UCL Institute of Child Health, London, UNITED-KINGDOM.<br/> (3) Max-Delbrück-Center for Molecular Medicine, Berlin, GERMANY.</p>  |
| To contact the author::<br>emartin@ibdml.univ-mrs.fr. | <p><i>Teashirt (Tshz)</i> genes encode transcription factors conserved between flies and mammals. We show that mouse ureteric smooth muscle cell (SMC) precursors express <i>Tshz3</i>, and that <i>Tshz3</i> null mutant mice have congenital hydronephrosis without anatomically-impaired urine flow. <i>In vivo</i>, failed ureteric muscle differentiation antedated urinary tract dilatation while <i>ex vivo</i>, wild-type but not mutant fetal proximal ureter segments contracted spontaneously. Moreover, the expression of myocardin, an essential component of the regulatory pathway for SMC differentiation, and of myocardin-dependent SMC genes was markedly downregulated in <i>Tshz3</i>-null mouse ureters prior to the onset of hydronephrosis. The data are consistent with a model in which <i>Tshz3</i> expression is required for SMC differentiation by pathways involving myocardin-dependent SMC transcription. These findings provide new insights into molecular pathways linking visceral SMC differentiation with muscle function, and they also emphasise the central role of the proximal ureter in the physiological transit of fetal urine from the kidney to the urinary bladder, with defects leading to congenital hydronephrosis</p> |

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| PW35-445   | <p><b><u>ROLE OF SRF IN THE RESPONSE OF VASCULAR SMOOTH MUSCLE CELLS TO MECHANICAL STRESS.</u></b><br/> GALMICHE G<sup>1</sup>, MERICKSKAY M<sup>1</sup>, BLANC J<sup>1</sup>, LI Z<sup>1</sup><br/> (1) UPMC/CNRS UMR7079, Physiologie et physiopathologie, Paris, FRANCE.</p>  |
| <p>To contact the author::<br/> guillaume.galmiche@snv.jussieu.fr.</p> | <p>Vascular smooth muscle cells (VSMCs) anomalies are directly involved in different forms of myopathies including Duchene myopathy and desminopathies. The alteration of cytoskeletal mechanotransduction and transcriptional pathways play an important role in the evolution of the vasculopathies. Serum response factor (SRF) is a key transcription factor in SMCs. The activity of SRF is modulated by the RhoA-actin pathway and growth factors signaling pathways. Our aim is to study the role of SRF in adult VSMCs <i>in vivo</i> and <i>in vitro</i> by conditional invalidation of SRF gene using a tamoxifen-inducible and smooth muscle-specific Cre recombinase, SM22-CRE<sup>ERT2</sup>(ki). In order to identify the targets of SRF in VSMCs <i>in vivo</i>, RNA was isolated from aorta 8 days after tamoxifen injection in control and mutant mice. Preliminary analysis of gene expression microarrays shows that more than 100 genes are differentially expressed in the aorta of the SRF mutants. The validation of these data by Q-PCR is ongoing. In order to evaluate the role of SRF in the transcriptional response of VSMCs to experimental cyclic stretch, we isolated VSMCs from the aorta of floxed SRF mice, and optimized the conditions for maintaining VSMCs differentiation in culture with a modified serum-free medium. We infected primary VSMCs cultures with a recombinant adenovirus expressing the Cre recombinase to inactivate SRF <i>in vitro</i>. More than 95% decrease of SRF mRNA expression was obtained by this approach. The effect of stretch on SMC-specific genes expression is currently evaluated using the FlexerCell system in control and SRF-deficient VSMCs. Chemical inhibition or dominant negative approach will be used to decipher the pathways linking biomechanical stretch to SRF activation.</p> |