showed that Fzd7 expression was modulated during the kinetic of EB differentiation and that Fzd7 was expressed in EC assembled in vascular structures. Fzd7 was highly localized in cell-cell junctions, colocalized with VE-cadherin and beta catenin-positive junctions.

In order to analyze the role of Fzd7 in endothelial cell function, effect of Fzd7 knock down by small interfering RNAs was investigated in human umbilical vein endothelial cells (HUVEC). KD of Fzd7 had no effect on cell proliferation whereas a strong inhibition of HUVEC migration and tube formation was observed. Indeed, Fzd7 siRNA decreased cell migration to 59±17% (n=3, p<0.0001) compared to control siRNA in transwell assay. We also found that HUVEC transfected with control siRNA form well-connected tubular structures in Matrigel assay after 18h, whereas Fzd7 siRNA treated HUVEC showed a strong delay in tubular structures formation (43±5 tubes/field in control vs 15±7 in Fzd7 siRNA, p<0.0001).

This finding suggests that Fzd7 may play a crucial role during vascular formation. Fzd7 is essential for zebrafish vascular development in vivo and regulates endothelial cells functions involved in angiogenesis in vitro.

D009
FRIZZLED 4 REGULATES VASCULAR FORMATION IN MICE

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A role for Wnt via the Frizzled (Fzd) cell surface receptors has recently emerged in vascular cell development. Among the Fzd members, Frizzled4 (Fzd4) was recently implicated in vessel formation in adult retinal vascular network. Consistent with this finding, we aimed to evaluate the function of Fzd4 in adult vascular setting.

MicroCT and immunohistochemistry approaches revealed that Fzd4 deletion altered dramatically coronary and arterial network formations in the heart and in the kidney with a significant decrease of the arterial branching. Micro-array analysis revealed that in hearts of Fzd4KO neonate mice, the primary effect of Fzd 4 deletion was the down-regulation of the cell cycle-regulating transcription factor E2F1, with a decreased expression of a large number of E2F target genes. This observation indicated that loss of Fzd4 lead to cell cycle arrest.

We conducted in vitro studies to confirm the role of Fzd4 on cell cyle response on murine embryonic fibroblasts (MEF) and endothelial cells (EC) isolated from KO Fzd4 vs Wt mice. Fzd4KO reduced MEF and EC proliferation. Fzd4 siRNA-mediated knockdown (KD) in EC delayed G1 to S cell cycle entry compared to that in control EC (transition G1 to S at 12H, 3,5% vs 14,5% vs control EC). In vitro matrigel assay showed that Fzd4 KD reduced the differentiation of HUVEC to form capillary structures under VEGF A-stimulation (43±4 in Fzd4 KD EC vs 28± 7, in control EC, P<0.005) but did not modify EC migration compared to control EC in a transwell migration assay.

These findings are the first to show that a frizzled member Fzd4 can repress E2F1 cell cycle progression in EC independently of the Wnt/β catenin canonical pathway and affects deeply vascular formation and branching.

D010
MESENCHYMAL STEM CELLS PROTECT CARDIOMYOCYTES FROM REPERFUSION INJURY THROUGH A PARACRINE ACTIVATION OF THE PI3 KINASE PATHWAY

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Objectives — Previous data suggest that implantation of mesenchymal stem cells (MSCs) improves heart function after myocardial infarction. We investigated whether protection afforded by MSCs might involve a paracrine activation of the PI3 kinase pathway in reperfused cardiomyocytes.

Method — MSCs and neonatal rat cardiomyocytes (NRCs) were isolated and cultured separately. NRCs (2.106) were subjected to 5 hours of ischemia followed by 16 hours of reperfusion. At the time of reperfusion, NRCs (n=8-14/group) received either fresh medium (control group), or the following treatments: MSCs (2.105 MSCs in fresh medium), conditioned SN (MSCs supernatant alone (i.e. without MSCs) obtained after 8 hours of serum deprived culture), [conditioned SN + LY294002] (15 microM of LY294002 a specific inhibitor of PI3K), [conditioned SN + Wortmannin] (100 nM of wortmannin, a non specific inhibitor of PI3K), or CsA (200 nM in fresh medium) a potent inhibitor of the mitochondrial permeability transition pore. Cell death was assessed by LDH release in NRCs supernatant at the end of reperfusion.

Results — As expected, LDH activity was dramatically reduced by CsA, averaging 4% of control values. LDH activity was significantly reduced by MSCs alone and by conditioned SN, averaging 29% and 12% of control value, respectively. Both LY294002 and wortmannin significantly attenuated conditioned SN induced protection.

Conclusion — our data suggest that MSCs can protect NRCs from reperfusion injury through a paracrine activation of the PI3K pathway.

D011
MESENCHYMAL STEM CELLS ENGINEERED TO OVEREXPRESS STEM CELL FACTOR IMPROVE CARDIAC FUNCTION BUT HAVE MALIGNANT POTENTIAL

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Background — Myocardial infarction (MI) in mice with mutations in the stem cell factor (SCF) receptor causes rapid heart failure. Implantation of mesenchymal stem cells (MSC) slows progression to heart failure after MI. We hypothesized that MSC engineered to over-express SCF would restore cardiac function better than unmodified MSC.