Methods Rat NSC line was isolated from the subventricular zone and purified using suspension culture technique as previously established in the lab. Rat NSCs were induced to differentiate into smooth muscle cells (SMCs) on type IV collagen coated flasks at 37°C. Cells were then harvested for mRNA and protein extraction at the 1st, 2nd and 4th day in differentiation medium. The mRNA and protein expressions of SMA, smooth muscle myosin heavy chain (SMMHC), smooth muscle protein 22 (SM22) and myocardin were detected using PCR and Western blot. Finally, the differentiating cells on the 4th day were prepared for immunofluorescent staining for SMA and SMMHC markers.

Results

1. Rat NSCs lost their neural shape from the 2nd day of differentiation and acquired a long, spindle-shaped phenotype and started to form a spiral structure on the flasks. On the 3rd day of culture, there was no significant difference between the differentiating Rat NSCs and wild-type SMCs.
2. PCR assays confirmed that the gene expression levels of smooth muscle cell-specific genes (SMA, SM22, SMMHC) and transcription factor (Myocardin) on the 2nd day were significantly increased ($p<0.05$) as compared to the expression in the 1st day. Similarly, the 4th day were significantly increased when compared with the 1st day and the 2nd day ($p<0.05$).
3. Western blot analysis confirmed that the protein expression levels of smooth muscle cell-specific genes and transcription factor gene (SMMHC, Myocardin) on the 2nd day were significantly increased ($p<0.05$) as compared to the 1st day. Moreover, on the 4th day of differentiation, all 4 genes expression were significantly increased as compared with the 1st and 2nd days ($p<0.05$).
4. More than 80% of the cultured cells were stained positive for SMA and SMMHC markers. The stainings showed characteristic shapes of the SMCs cytoskeleton, which connects with the cell membrane system extensively.

Conclusions Rat NSCs were induced successfully to differentiate into SMCs on type IV collagen coated flasks at 37°C that has been demonstrated by the expressions of specific SMCs markers. To date, this is the first report that Rat NSCs could differentiate into SMCs. Our results indicate the possibility that neural stem cells could take part in *in vivo* angiogenesis, which would promote neural stem cell transplantation as a future therapeutic alternative for ischaemia neural tissue.

GW23-e2203

INDUCED DIFFERENTIATION OF RAT NEURAL STEM CELLS TOWARDS SMOOTH MUSCLE CELLS IN VITRO

doi:10.1136/heartjnl-2012-302920b.7

[1]Peng Liyuan, [1]Yan Bin, [1]Su Dan, [1]Wei Jin, [1]Dong Xin, [2]Zeng Lingfang, [1]Wang Gang, [1]Wang Gang. *[1]Department of Cardiology, The Second Affiliated Hospital, Xi'an Jiaotong University School of Medicine, Xi'an 710004, Shaanxi, China; [2]Cardiovascular Division, King's College London, James Black Centre, London SE5 9NU, England*

Objectives Based on our pilot data, transplanted rat neural stem cells (NSCs) adhered to the vascular basement and began to express levels of smooth muscle α actin (SMA) in vivo. This current study is to investigate the differentiation of NSCs towards smooth muscle cells in vitro.