Cardiovascular stem cell biology and development

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Abstract

The proliferative potential of pluripotent stem cell derived cardiomyocytes is limited and reasonable yields for novel therapeutic options have yet to be achieved. In a first attempt of such “cardiovascular forward programming” using pluripotent stem cells, we have previously shown that MesP1 represents a master regulator sufficient to induce cardiovasculogenesis (David et al., Nat Cell Biol, 2008). In ES cells MesP1 overexpression resulted in significantly increased numbers of beating cardiomyocytes and of endothelial cells. Our experiments revealed a prominent function of MesP1 within a gene regulatory cascade causing Dkk-1 mediated blockage of canonical wnt-signalling. Our findings suggest a mechanism for cardiovascular specification highly conserved in vertebrates initiated via MesP genes with prominent factors such as Nkx acting further downstream. Detailed patch clamping analyses showed electrophysiological characteristics corresponding to all subtypes of cardiac ES cell differentiation in Nkx2.5 as well as MesP1 programmed embryoid bodies (EBs) but fractions of cardiomyocytes had distinct characteristics: MesP1 forced the appearance of early/intermediate type cardiomyocytes (~60%) in comparison to control cells whereas Nkx2.5 led to preferentially differentiated ventricular cells (~80%) (David et al., Cardiov Res, 2009). In order to unravel the regulation of MesP1 expression we have now analysed Eomes and Brachyury(T) as its potential inducers. We demonstrate that the MesP1 positive cell population is derived from the Brachyury(T) positive fraction in the embryo as well as in ES cells. Likewise, loss of Brachyury(T) causes a dramatic decrease of MesP1 expression accompanied by reduced cardiac markers. Using EMSA, ChIP and reporter assays we found a 3.4 kb proximal MesP1 promoter fragment, directly bound and activated by Brachyury(T) via a T responsive element (David et al., Cardiov Res, 2011). To characterize the cellular progeny eliminating an overexpression situation we used this promoter fragment for isolating MesP1 positive cells from differentiating pluripotent stem cells via magnetic cell sorting based on a deleted CD4 surface marker. This yielded a highly pure common cardiovascular progenitor population with the potential to form all three cardiovascular lineages: cardiomyocytes, endothelial cells and smooth muscle cells. Electrophysiological and pharmacological parameters of the derived cardiomyocytes affirm the pivotal role of MesP1 during the earliest cardiovasculogenic events: by far most of the cardiomyocytes (~94%) corresponded to the desirable multipotent early/intermediate type highly exceeding the numbers achieved by the above described forward programming via MesP1 (~60%) (David et al., in revision).

Finally, I will address ongoing work transferring our forward programming approach described above to the enrichment of pacemaker cells, which may become useful for biological treatment of the “sick sinus syndrome”.