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EDITORIAL

Adrenergic function restoration in the transplanted heart: a role for neural crest-derived cells

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This editorial refers to 'Neural crest-derived resident cardiac cells contribute to the restoration of adrenergic function of transplanted heart in rodent' by Y. Tamura *et al.*, pp. 350–357.

Cardiac transplantation is currently the best therapeutic approach for some end-stage heart failure patients.[1] One consequence following cardiac transplantation is cardiac denervation due to the severance of post-ganglionic sympathetic nerve fibres.[2] Interestingly, sympathetic reinnervation takes place within a year or more following transplantation.[3] This is accompanied by a significant improvement of heart rate and function in response to exercise, as well as improvement of coronary blood flow.[3,4] This recovery of adrenergic function in the transplanted heart has been attributed in part to the activation of the intrinsic cardiac adrenergic system, which can stimulate adrenergic function independent of sympathetic innervation.[5,6] Identifying the origin and the mechanisms of activation of the intrinsic cardiac adrenergic system could shed light on sympathetic-related events such as ventricular arrhythmias.[7]

In this issue, Tamura *et al.*[8] aimed to identify the cellular origin of the intrinsic adrenergic system, which gets activated after heart transplantation. The authors hypothesized that neural crest-derived cardiac resident populations in the heart contribute to neuronal remodelling and adrenergic function activation following transplantation. To address this question, the authors used transgenic mice expressing Protein 0 Cre and an EGFP reporter, to label neural crest cell populations that express Protein 0 with EGFP. The EGFP signal was detected as early as E10.5 in the mouse heart, and a subpopulation of these cells were nucleated and expressed tyrosine hydroxylase, which is a catecholamine biosynthesis enzyme. This indicated that neural crest-derived tyrosine hydroxylase-positive cells are present before sympathetic innervation takes place. Following sympathetic innervation, the number of these cells was significantly reduced, but they were still present in the adult 8-week-old heart.

To determine whether neural crest-derived cells contribute to the activation of the intrinsic adrenergic system following heart transplantation, the hearts from the Protein 0-Cre/floxed EGFP transgenic mice were transplanted heterotopically in NOD-SCID mice. Sympathetic nerve fibres were absent from the transplanted heart due to the denervation associated with transplantation. Interestingly, there was a significant increase in the number of EGFP-positive neural crest-derived cells within a week following transplantation. However, the tyrosine hydroxylase-positive cells only increased in number within 2 weeks following transplantation. This was associated with an increase in the levels of nerve growth factor (NGF), which occurs after sympathetic denervation after heart transplantation. Furthermore, isolated neural crest-derived cells proliferated and differentiated into tyrosine hydroxylase-expressing cells in response to NGF *in vitro*. This suggests that NGF stimulates the expansion and differentiation of neural crest-derived cells after transplantation.

Adrenergic function in the heart can be determined by measuring the expression levels of catecholamine-synthesizing enzymes. Within a week after transplantation, the authors detected a reduction in the levels of catecholamine-synthesizing enzymes, as expected due to denervation. These levels started to increase gradually by 2 weeks after transplantation. This was confirmed by measuring the transplanted heart content of catecholamines by chromatography. Thus, the increase in catecholamine levels coincided with the increase in the number of tyrosine hydroxylase-positive neural crest-derived cells.

Catecholamine uptake and storage is a hallmark feature associated with the ability of the transplanted heart to exhibit improved ventricular inotropic and chronotropic responses to exercise.[3] The authors measured catecholamine uptake in the transplanted heart by iodine-123-MIBG. Similarly, catecholamine uptake was reduced 1 week after transplantation and started to increase by 2 weeks, at the time point when tyrosine hydroxylase-positive neural crest-derived cells increased. This further suggests the correlation between tyrosine hydroxylase-positive neural crest-derived cells and increased catecholamine uptake and storage.

Finally, to determine restoration of adrenergic function in the transplanted hearts, the authors injected tyramine for stimulation of intracellular catecholamine secretion, which leads to an increase in heart rate with normal adrenergic function. No increase in heart rate was detected within a week after transplantation, while heart rates increased only 2 weeks after transplantation. To further confirm that this effect is

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due to the restoration of the intrinsic adrenergic system and not due to other exogenous effect, the transplanted hearts were perfused and treated with tyramine *ex vivo*. Similarly, the heart rates of the transplanted hearts did not increase in response to tyramine within 1 week, but only after 2 weeks did the heart rates start to increase. Thus, restoration of the intrinsic adrenergic function of the transplanted heart occurs during the increase of the tyrosine hydroxylase-positive neural crest-derived cells, which suggests that the tyrosine hydroxylase-positive neural crest-derived cells mediate this response.

This interesting study identifies for the first time a strong association between neural crest-derived stem cells and the intrinsic cardiac adrenergic system, which gets activated following sympathetic denervation.[8] It is intriguing that the sympathetic nervous system exclusively regulates adrenergic function in the adult heart, while the intrinsic adrenergic system gets activated in the event of denervation. A major question to be answered is whether the neural crest-derived tyrosine hydroxylase-expressing cells exclusively modulate the intrinsic cardiac adrenergic function. Thus, it would be important to deplete these cells and determine whether this abolishes the restoration of adrenergic function, thus confirming that these cells are necessary for the intrinsic cardiac adrenergic function. Furthermore, identifying the source of NGF following denervation would increase our understanding of the mechanisms of activation of neural crest-derived tyrosine hydroxylase-positive cells, which would be an important step towards harnessing the full potential of these cells for fine tuning the adrenergic system in the heart.

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