Journal of Genetic Syndromes & Gene Therapy
ISSN: 2157-7412

Zhang et al., J Genet Syndr Gene Ther 2013, S3
DOI: 10.4172/2157-7412.S3-008

Review Article Open Access

Adult NG2-Expressing Cells in Multiple Organs: A Novel Progenitor in Regenerative Medicine

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Abstract

Stem/progenitor cells have emerged as a promising tool for studying the mechanisms of cell development, tissue regeneration, and cell therapy for various disorders. Stem/progenitor cells are found not only in the embryo but also in most adult tissues for endogenous repair. They are capable of self renewal and differentiation into various cell types from their germ line, thus, are ideal candidates for cell-based therapy. Expression of neuron-glial antigen 2 (NG2) proteoglycan is found on several types of cell surface, majorly distributing on undifferentiated precursor cells in the Central Nerve System (CNS), named Oligodendrocyte Progenitor Cells (OPCs). NG2 proteoglycan has a widespread range of physiological roles and the cells that express chondroitin sulphate proteoglycan NG2/CSPG4 ($NG2^+$cells) react to all forms of pathological insults. This cell population is abundant in the developing and mature organs with also pericytes PCs) and mesenchymal stem cell (MSCs) potential. Our pilot studies demonstrated that $NG2^+$cells are dedicated stem/precursors not only in the CNS as traditional thought, but also outside the CNS. Following injury, the repertoire of $NG2^+$cells expands to become functional cells. Our experiment also showed the importance of $NG2^+$cells as the reservoir for MSCs, which reaffirms the central role of these cells for their therapeutic potential. In this review, we provided some pilot evidence and briefly summarized the more recent progress on adult $NG2^+$cell researches. We hypothesis that adult $NG2^+$cells can be generated from not only the adult mammalian's CNS but also multiple outside organs including spinal cord, bone marrow, eyes, liver, heart, lung, pancreas and kidney. Therefore, development and research of adult $NG2^+$cells may open a novel perspective in regenerative medicine in the future.

Keywords: NG2-expressing cells; Self-renewal; Tissue repair; Pericytes; Mesenchymal stem cells

Abbreviations: BM:MSCs: Bone Marrow: Derived Mesenchymal Stem Cells; BrdU: Bromodeoxyuridine; CNS: the Central Nervous System; EC: Endothelial cells; ECM: Extracellular Matrix; ERK1/2: The Extracellular Signal: Regulated Kinase ½;GABA:A: Gamma-Aminobutyric Acid-A; GFAP: Glial Fibrillary Acidic Protein; GluRs: Glutamate Receptors; GR: Glutamate Receptors; GS: Glutamine Synthetase; HSCs: Hepatic Stellate Cells; IC: Ischemic Cardiomyopathy; MAPK: Mitogen Associated Phosphate Kinases; MI: Myocardial Infarction; MS: Multiple Sclerosis; OPCs: Oligodendrocyte Precursor Cells; PC: Pericytes; PDGFR-β: Platelet-derived Growth Factor Receptor-Beta; α:SMAs: Alpha Smooth Muscle Actin; SMCs: Smooth Muscle Cells; Tkase: Tyrosine Kinase; Vwf: Von Willebrand Factor

NG2-Expressing Cells are Progenitors in the Adult Central Nervous System

The story of NG2 proteoglycan being as a valuable marker for identification of oligodendrocyte precursor cells (OPCs) in the Central Nervous System (CNS) began nearly thirty years ago [1]. Since then, scientists have postulated that NG2 proteoglycan expression might be the characteristic of immature neural cells capable of differentiating into either glia or neurons, hence the designation of the molecule as "nerve/glial antigen 2" [2]. However, the function of NG2-expressing cells ($NG2^+$cells) in the adult CNS is still uncertain although that the $NG2^+$cells are progenitor cells in the developing CNS is well documented [3]. In addition, the contribution of functional receptors of $NG2^+$cells on neurons in response variety of injuries indicates $NG2^+$cells plays a significant role in stimulating neural network as well as their potential importance in inflammation, neurodegeneration and regenerative medicine [4].

Phenotypic and physiological roles of $NG2^+$cells in the adult CNS

Cells expressing the chondroitin sulphate proteoglycan NG2/CSPG4 are glia lineage which makes up 5-10% of all glia in the adult CNS. The morphology and distribution of NG2 proteoglycan are similar to, but distinct from, both microglia and astrocytes. The $NG2^+$cells are present early in development and persist ubiquitously throughout CNS parenchyma where they exhibit multi-branched processes, big cell bodies and small nuclear. NG2 proteoglycan is a type 1-transmembrane protein expressed by a range of cell types within the mammalian nervous system. They are found in grey and white matter tracts of the developing and adult CNS. In vitro $NG2^+$cells possess some functional receptors and contact neurons at the nodes of Ranvier or via synaptic terminals and some of them even have fire action potential. There has recently been much debate concerning the possible heterogeneous nature of the $NG2^+$cells with respect to developmental

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Received April 16, 2013; **Accepted** May 06, 2013; **Published** May 09, 2013

Citation: Zhang H, Zhang X, Bie P, Miller RH, Bai L (2013) Adult NG2-Expressing Cells in Multiple Organs: A Novel Progenitor in Regenerative Medicine. J Genet Syndr Gene Ther S3: 008. doi:10.4172/2157-7412.S3-008

origin and functional roles. The possibility of multiple and varied functions of NG2$^+$cells also suggest that such cell population may be heterogeneous [5]. Several studies have proposed that there may be two distinct populations of NG2$^+$cells in the developmental CNS, one is able to generate mature oligodendrocytes whereas the other has undefined roles [6-8]. For example, researchers have examined the response of NG2$^+$cells following demyelination in the rat spinal cord and identified the presence of two NG2$^+$cells populations, one responding to the insult by dividing and the other non-responsive, suggesting that the responsive population acts as oligodendrocyte progenitors [9]. However, whether the NG2$^+$cells residing in the adult CNS resemble to the ones in embryonic or neonatal CNS in terms of their morphology or proliferation characteristics remains unsolved [10].

Defining the role of adult NG2$^+$cells *in vivo* CNS has remained elusive despite their phenotypic characterization as OPCs and are able to give rise to oligodendrocytes *in vitro* [11,12]. NG2 molecule posses a large extracellular domain (300 kDa core protein) and was initially identified by a sequentially-absorbed rabbit antiserum [13,14]. The big molecule of NG2 includes sites near the single transmembrane domain which are readily cleaved by a variety of proteases [15], resulting in the deposition of the ectodomain in the extracellular matrix (ECM). Although having relatively slow rate of cell division adult NG2$^+$cells represent the major proliferative population in the adult CNS, accounting for 70% and 74% of bromodeoxyuridine (BrdU)-incorporating cells in the spinal cord and cerebral cortex respectively [16,17]. Generation of newly oligodendrocytes from adult CNS is an expected function, but it is likely that they also participate in homeostasis, glutamate signaling and interdigitate between pre- and post-synaptic terminals [18,19]. Experiments in some laboratories identified a serological epitope on human melanoma cells known as HMW-MAA [20-22] as the human homolog of NG2 proteoglycan [23]. Subsequently, detected excitatory synapses mediated by AMPA receptors on NG2$^+$cells and found respond to neuron released glutamate of such cells [18], indicating the expression of glutamate receptors (GR) on NG2$^+$cells. Furthermore, the presence of GR on NG2$^+$cells in the adult CNS regions has been recognized and reported as functional gamma-aminobutyric acid-A (GABA-A) receptors [24]. This is more likely to act as a rapid means of altering ion concentrations within NG2$^+$cells [25,26], thereby altering their physiological properties. In keeping with a role in glutamate neurotransmission suggesting oligodendrocytes and NG2$^+$cells are both to express glutamine synthetase [16,17]. Thus, there is good evidence for a role for NG2$^+$cells in glutamine cycle.

Stem/progenitor potential of NG2 cells for neurological disorders

NG2 proteoglycan can be detected in a variety of CNS diseases including demyelinating diseases of which multiple sclerosis (MS) is the best studied. Understanding the mechanisms underlying remyelination is essential to elucidate the reasons for the development of chronic demyelinated plaques seen in MS. In experimental models of demyelination, remodeling of NG2$^+$cells for denuded axons is usually an extremely rapid and efficient repair process, regardless of the method of induction of demyelination [27,28], suggesting NG2$^+$cells are largely responsible for myelin repair [29]. It has been demonstrated that cell proliferation is required in order to replenish lost oligodendrocytes [30] and the expression of Ki-67 antigen adult NG2$^+$cells in the presence of an toxicity indicating proliferation and undergo morphological changes of this cell population [15]. In human MS tissues, the antigenic profile and morphology of NG2$^+$cells are consistent with an oligodendrocyte progenitor function. By using retroviral labeling as tracking tool demonstrated that NG2$^+$cells are the major cycling cell in the adult CNS not just least the progenitors of oligodendrocytes when cultured them in vitro [16,17].

Adult NG2$^+$cells will undergo well-characterized reactive changes in response to CNS pathology. Adult NG2$^+$cells has been tested by various CNS injury models and found that grafted cells adjacent to the damage site where increase in number and become hypertrophic [7,31,32]. A study reported that NG2$^+$cells generate mature oligodendrocytes, in transgenic mice neurons alone with subpopulations of astrocytes during normal development, indicating their stem/progenitor potential. Therefore, a new method is to be required for the development of NG2$^+$cell isolation or purification. Based on this line, we purified NG2$^+$cells for the first time from adult mouse spinal cord by using a novel procedure (Figure 1) and found that this purified NG2$^+$cells are highly homogenous (more than 98% of cells express NG2 proteoglycan). They display discoid, heterochromatic, irregular shape with multiple processes and importantly to response to injury. For instance, these transplanted cells could move into diseased conditions, protecting neural cell survival as well as increase remyelination in a mouse model of demyelinating disease such as in Experimental Allergic Encephalomyelitis (EAE) [33]. In addition, our study provided strong evidence that NG2$^+$cells are the highest dividing cell population comparing to other glial cell types when their receiving damage signals. Such phenomena were consistence with a study from Lee et al. laboratory. They have reported that injection intravenously of

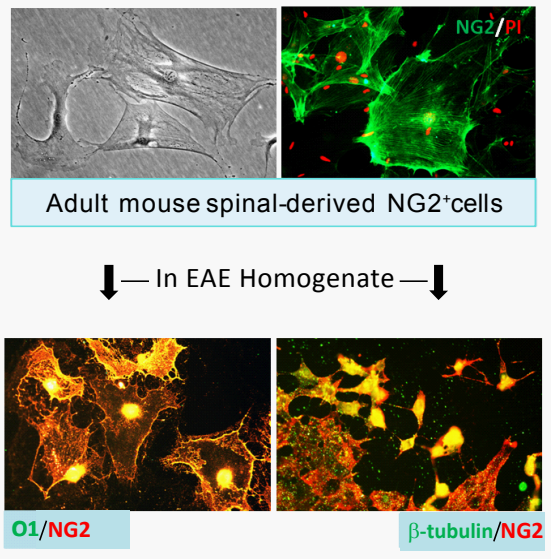

Figure 1: Mouse NG2$^+$cells from adult spinal cord can differentiate into mature oligodendrocytes and neurons. Spinal cords from two C57Bl/6J mice (> 8 wks) were dissected in Modified Eagle's Medium (MEM) on ice. After surface blood vessels were carefully removed, the tissue was digested for 30 min and then spun at 800 x g for 5 min. The pellets were washed with PBS for 3 times and layered on a Percoll gradient. Following centrifugation at 1,100 x g for 30 min, the cellular fractions were collected, washed and resuspended in DMEM medium containing 10% FBS at a density of 1 x 10^6 cells/mL in a 75 cm^2 flask. The cells grew at 37°C under 5% CO_2 for at least 4 weeks with a change of 50% medium every 2 days. Cells were passaged at least once prior to experiments, and 99% of cells were NG2 positive whereas no detectable mature oligodendrocytes expressing myelin markers CNP or MBP were found in the cultures and the number of GFAP$^+$astrocytes was less than 3%. Representative images show that the purified NG2$^+$cells were cultured in the presence of homogenates derived from spinal cord of EAE mice (demyelinating signals), and found differentiated into O1$^+$ mature oligodendrocytes and β-tubulin III$^+$ neurons.

NG2$^+$cells resulted in grafted cells migrated into the lesion areas where they attenuated the lesion atrophy and induced a long-term functional improvement in an animal model [34]. Since differentiation potential is thought to be influenced by NG2$^+$cell environment *in vitro* [35], it is possible NG2$^+$cell may display a broader lineage potential following CNS disease [13,36]. They may participate in tissue repair in response to injury and promote regeneration in demyelinating insult by generating further differentiated functional oligodendrocytes and neurons. More importantly, these purified adult CNS-derived NG2$^+$cells (CNS-NG2$^+$cells) could be also applied in transplantation therapies for treating diverse other organ disorders such as eye diseases.

Are NG2-Expressing Cells Progenitors in the Adult Optic Nerve?

The optic nerve is a CNS white matter tract that connects eye to brain. It contains glia, astrocytes and axon of retinal ganglin cells. Among glia, the major populations are oligodendrocytes, astrocytes and microglia as well as NG2$^+$cells.

Physiological and pathological functions of NG2$^+$cells in adult optic nerve

In the developmental optic nerve, NG2$^+$cells are highly identified in grey and white matter [37]. NG2$^+$cells are a small branch originated in the row of oligodendrocytes that make connection with the nodes of Ranvier in white mater and function (synapse) in grey matter [18]. In the adult optic nerve, only 7% of NG2$^+$cells exist glial cells and their composition are only limited within optic nerve head in demyelinating lesion. NG2$^+$cells share some similarity with astrocytes in morphology and biological characteristics [18] but do not express astrocyte markers like GFAP, vimentin, calcium-binding protein S-100b and glutamine synthetase (GS) [38].

It is well known that the cells expressing NG2 in the CNS were considered as OPCs [10], because of their being able to develop into oligodendrocytes or oligodendrocytes-type-2 astrocytes (O-2A) progenitors *in vitro* [39]. It is demonstrated that the stage of generating newly oligodendrocytes from NG2$^+$cells in developmental CNS [40-42] would express transaction marker, a tetraspanin protein CD9 [43], while CD9 was lost on NG2$^+$cells due to remarkable decreased proliferative rate in the optic nerve [41,44,45]. However, much work has been done for the differentiation of oligodendrocytes from NG2$^+$OPC [46,47], but the problem of whether the newly NG2$^+$OPCs differentiated oligodendrocytes have function remains opened.

NG2$^+$cells in the adult optic nerve are regenerative stem/progenitors

The presence of NG2$^+$cells in the optic nerve may have implications for efforts to enhance endogenous repair in a wide range of disorders [27]. However, the factors that regulate the diverse responses of NG2$^+$cells in optic nerve to damage are elusive. Several lines of evidence indicate a potential role of NG2$^+$cells in optic nerve for the neurotransmitter glutamate [48]. Firstly, NG2$^+$cells express AMPA-type glutamate receptors (GluRs) [18,49] by axons in white matter [50,51]. Secondly, there is evidence that AMPA receptors on NG2$^+$cells are permeable to Ca^{2+}[51-53], and this study provided a mechanism by which glutamate could regulate intracellular pathways that control cell proliferation, growth, differentiation and death, such as the calcium-dependent mitogen-associated phosphate kinases (MAPK), extracellular signal-regulated kinases (ERK1/2). Finally, glutamate has a well-defined role in the pathology of oligodendrocyte lineage cells [54]. Therefore, NG2$^+$cells like NG2$^+$OPCs and oligodendrocytes are extremely susceptible to glutamate-mediated calcium-dependent excitotoxicity [49,55]. Some experimental results showed that injection of the potent glutamate receptor agonist (kainite) into the cerebral cortex and white matter of the optic nerve induced an extensive gliosis [19,56] and caused the loss of oligodendrocytes demyelinating injury [56,57].

It is very important to address whether existed NG2$^+$cells in optic nerve have comparable biological feature with CNS-NG2$^+$cells, we used the same procedure as adult CNS-NG2$^+$cells and purified successfully NG2$^+$cells from adult mouse eyes (Iris) (Iris-NG2$^+$cells). Our experimental results showed that NG2$^+$cells have similar morphology and biological characteristics with CNS-NG2$^+$cells. They exhibit a more polarized appearance, radial morphology, extending processes and fine multi-branching processes and lack expression of GFAP [43]. Iris-NG2$^+$cells involved in a number of crucial cellular processes. When grown them in the injured conditioned media (retinal homogenenate) collected from retinal pigment mutation mice with tyrosine kinase (TKase) mutation (lack TKase function), the purified Iris-NG2$^+$cells differentiated into Opsin+ functional cone neurons (Figure 2).

Are NG2-Expressing Cells Progenitors in the Adult Bone Marrow?

The contribution of NG2$^+$cells to tissue repair in brain and eye disorders has been highlighted in recent studies, but far from being restricted NG2$^+$cells as progenitors is OPC marker. It is also an effective progenitor marker in bone marrow (BM) [58] existing as pericytes (PCs) or mesenchymal stem cells (MSCs) [59,60]. We propose the existence of these "adult NG2 progenitors" in BM may immediately recognize to have important implications for tissue repair during

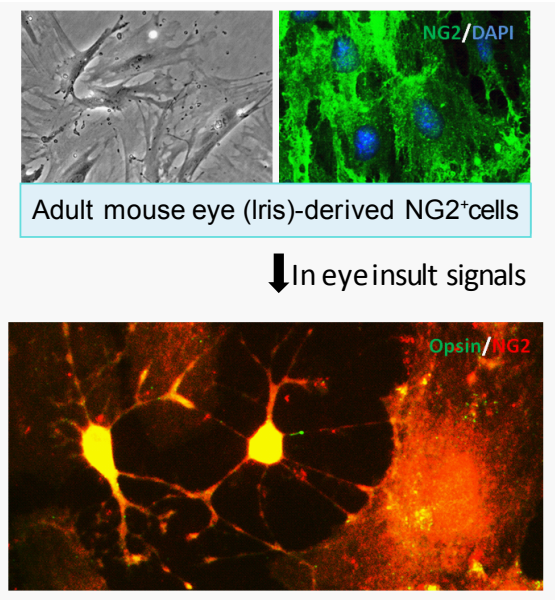

Figure 2: Mouse eye (Iris)-derived NG2-expressing progenitors (eye (Iris)-NG2$^+$cells) differentiate into functional Opsin+core neuron in response to injured eye homogenates. Mouse eye Iris from two C57Bl/6J mice (> 8 wks) was used to obtain NG2$^+$cells. The rest of procedures to isolate NG2$^+$cells were performed as described above. NG2$^+$cells were cultured in the presence of homogenates derived from eye injured mice. Representative images show that the isolated NG2$^+$cells differentiated into functional Opsin+core neuron when cultured in the presence of homogenates derived from Iris mice (eye insults).

diseases of diverse tissues. Hopefully, an increase in the pace of our acquisition of knowledge about the stem/progenitor functional role played by adult NG2⁺cells in both normal and pathological tissues for regenerative medicine will be seen in the coming years.

Are NG2⁺Cells the reservoir for mesenchymal stem cells?

Much of the work conducted on adult stem/progenitor cells has focused on mesenchymal stem cells (MSCs) which is a leading candidate stem cell population for clinical applications. Adult MSCs can be isolated from a range of stroma tissues including bone marrow (BM-MSCs) and fat [61]. One capacity of MSCs that is well established is their ability to release a wide range of cellular modulators that can modulate the host tissue [62] and immune responses [63-65]. The capacity of BM-MSCs modulate immune response combined with the release of a range of trophic factors contributing injured tissue regeneration, thus MSCs have being brought into a broader therapeutic scenario. Recent studies have demonstated that MSCs can be generated from NG2-expressing PCs within injured area and create a regenerative environment by releasing bioactive trophic factor. For example expression of NG2, PDGF receptor beta (PDGFR-β) and α-smooth muscle actin (α-SMA) [66], strongly supporting MG2-expressing MSCs possess PC potential. The term "MSCs" was first used to describe as adherent, marrow-derived homogeneous cells. They can divide in *ex vivo* and generated a diversity of connective cells [67]. Although a body of studies verified that BM-MSCs can differentiate along mesodermal lineages to form bone muscle and cartilage [68] the mechanism underlying their differentiation is not clear. There are detailed and elegant studies in the literature to support the fact that MSCs can be observed for almost every perivascular locations (on both arterial and venous vessels) and PC antigens can be identified by anti-NG2 and anti CD146 antibodies [69,70].

More work has been done about BM-derived NG2⁺cells but still no conclusion can be drawn regarding the real foothold of PCs and MSCs, and also the relationship between NG2-expresing PCs and MSCs remains to be established, there are several open questions: (1) Can NG2-expressing MSCs be named PCs and how to picture NG2-expressing MSC progenitor potential? (2) If NG2⁺cells were truly BM-MSC progenitors, can NG2-expressing MSCs be functional as vascular PCs? (3) Do the multiple organ-derived NG2⁺cells share the same biological characteristics? (4) are NG2-expressing PCs from bone marrow 'better' than the ones from other organs?

The challenge of translating bench studies on BM-NG2⁺MSC therapies to the bed application

The last decade has seen a remarkable advance of BM-MSCs therapeutic benefit from studies on animal models and pre-clinical investigations. The disorders and conditions appeared to benefit from infused BM-MSCs result from two generalizable therapeutic activities, immunomodulation and trophic activities [71]. Immunomodulation involves dendritic cells, B- and T-cells [72-75], while trophic activities involve MSC-released bioactive factors that shift the inflammatory Th cells from Th1 to Th2 [76], prohibit inflammatory cytokine production, scar formation [77-79], and angiogenesis [80], resulted in tissue repair by stimulating intrinsic progenitor cell activation, proliferation and appropriately differentiate [62,81].

A major challenge in moving MSC therapies into the clinic is to understand the mechanism that MSC existed. In this regard, encouraging our current researches came from the study on animal models of MS and demonstrated that human BM-MSC-secreted hepatocyte growth factor (HGF) is a critical molecule that contributed to immunomodulatory and direct regeneration. Treatment of mice with EAE and lysolecithin (LPC)-induced demyelination with human MSC-HGF contained conditioned media (MSC-HGF-CM) resulted in a dramatic reduction in functional deficits, improved histological appearance and alteration in the relative levels of pro and anti-inflammatory cytokines [82]. However, current knowledge regarding the immunobiology and clinical application of BM-MSCs or HGF need to be strengthened further to establish BM-MSCs or HGF as effective therapeutic tool in regenerative medicine. A further useful detail of BM-MSCs with particular reference to their derivation from NG2-expressing PCs and their potential for possible use in tissue regeneration and repair warrants further study. At present, we are working on a critical issue: Can NG2⁺cells purified from BM (BM-NG2⁺cells) generate newly MSCs?

To address this question, it is a considerable value to purified NG2⁺cells from adult mammalians BM. we have used the same procedure as CNS-NG2⁺cells and eye (Iris)-NG2⁺cells and purified this cell population. We found that NG2-expressing cells can be purified from BM. Based on our experiments we presented a remarkable outcome. As in images showed (Figure 3) that approximately >95% of BM isolated cells express NG2 proteoglycan (BM-MG2⁺cells) and performed not only self renewal but also differentiated into CD90-expressing

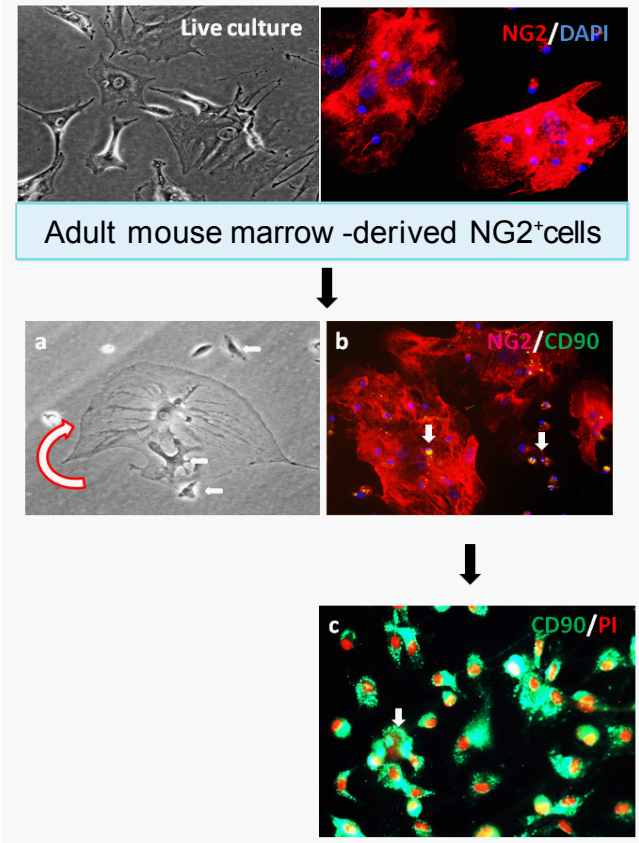

Figure 3: Mouse bone marrow-derived NG2-expressing cells (BM-NG2⁺cells) exhibit self-renewal and yield CD90⁺ mesenchymal stem cells (MSCs). Mouse bone marrow from two C57Bl/6J mice (> 8 wks) was used to obtain NG2⁺cells. The rest of procedures to isolate NG2⁺cells were performed as described above. Representative images show that mouse bone marrow-derived NG2-expressing cells exhibited self-renewal and yield CD90⁺ MSCs.

MSCs. We propose that these cells may like NG2$^+$cells located other organs have local functions in the tissue microenvironment beyond mesenchymal differentiation and functional outcome in response to insult signals. This study strongly supported our hypothesized: BM-NG2$^+$cells are the reservoir of MSCs. The NG2-derived MSCs could secrete a variety of beneficial factors that modulates immune response and promote endogenous regeneration. We also proposed that the possibility of a reservoir of NG2-expressing PCs for MSCs that would have the similar biological and functional capacities that survey tissue repair [69,83-87].

Are NG2$^+$Cells from Outside CNS Organs the Originators in Tissue Repair?

Similarity of functional properties of NG2$^+$cells, MSCs and PCs exist in multiple diseased organs is entirely unclear. The MSCs isolated from multiple adult organs proliferate extensively in the cultures of unselected. PCs, as a monolayer of dendritic-like cells closely ensheath endothelial cells within capillaries and microvessels (arterioles and venules), have been recently identified as possible originators of MSCs [88]. It is clear that purified perivascular cells express NG2 which exhibit multiple mesodermal developmental potentials, and become indistinguishable from conventionally derived MSCs after *in vitro* culture [89]. However, the possible roles played by these blood vessel-bound NG2-expressing PCs and MSCs in organogenesis and adult tissue repair remain elusive. Better knowledge of the lineage affiliation of these cells will contribute to the development of more efficient and tissue repair.

Adult cardiovascular NG2$^+$cells in heart failure repair

Homeostasis of different cell types in cardiovescular wall is essential for maintaining heart function. In physiological conditions, the turnover rate of vascular cells is low but greatly increased in diseased situations, e.g. vascular injury after angioplasty. It is believed that mature vascular cells have an ability to proliferate to replace lost cells and recent evidence indicates stem/progenitor cells may participate in vascular repair in damaged vessels.

In general, there are three layers (intima, media, and adventitia) of immature cardiovascular vessels. Some progenitor cells also reside in cardiovascular vessel such as endothelial progenitors, MSCs, Sca-1$^+$, CD34$^+$ and NG2$^+$cells [90-92]. These resident progenitor cells quietly persist in adult healthy heart and waiting for the opportunity for cell therapy in response to injury signals. Below we discuss the data suggesting the presence of NG2-epressing cells that reside in the adult heart exhibit PCs characteristics. Mature cardiovascular vessels have three cellular parts: PCs, endothelial cells (ECs) and smooth muscle cells (SMCs) that have developed independently during development [93]. After maturation, the role of progenitors in healthy and pathological processes has been well documented in different animal models [94,95]. It takes long time to understand how those progenitors contributed to vascular homeostasis, proliferation, angiogenesis and tissue repair. An interesting study recently showed that PCs being as progenitors that response to local injury by differentiating into functional cells [96].

Several aspects contribute to defining a suitable source of MSCs for treatment of different insult conditions. Tissue injury may require relatively few cells places in a specific location adjacent to the area of damage, while PCs in the wall of blood vessels may require damage signal stimulation to give rise to MSCs. A study suggested that aortic arch is the major source of PCs and aortic arch-derived PCs express NG2 [97]. Further study revealed that NG2$^+$cells that isolated from aorta arch were provided with vasculogenesis, suggesting PC potential [98]. Notably, PCs jointing with capillary ECs, indicating NG2-expressing PCs can be regarded as the structural components of blood vessels to regulate vascular contractility and perform functional task through generated MSCs [60,70,99-102]. We propose NG2 expressing PCs cells in adult heart blood vessel are an attracted cells. They may endue characteristics with PC and generate MSCs in response to insult with broad developmental potential for heart failure [103].

PCs distribute not only in veins but arteries as well. They do not express markers of hematopoietic stem cells (CD34, CD45) and endothelial cells (vWF) [104]. A study showed that cultured a cell population that generated from rat aortic in a collagen and bFGF containing conditioned media, the cells formed spheroid colonies and cell surface antigens were identified by NG2, PDGFR-β and nestin antibodies [105], suggesting cardiovascular vessel wall process PC-like NG2$^+$cells. Recent histological observations showed that blood vessel wall-derived NG2-expressing PCs can also differentiate into adipocytes, chondrocytes, osteoblasts within calcified atherosclerotic lesions [106,107], suggesting their mesenchymal origin and adipogenic and osteogenic potential.

NG2-expressing PCs per se serve as blood vessel progenitor cells [108,109] and can generate cardiomyocytes, smooth muscles and vascular EC in homeostatic conditions and after myocardial injuries for constitution of myocardium within the adult heart. Isolation and stimulation of PCs in adult heart may represent a potential therapeutic strategy to treat heart failures [110-112]. Consistent with this notion a number of studies of cardiac injury in animal model have suggested the clinical potential of heart-derived NG2-expressing PCs as progenitors in regeneration following cardiac injury [113]. Another study showed that grafted NG2-expressing cells that isolated from blood vessels into animal model, improved cardiac fabric construction and function through the combined effect of myogenesis and angiogenesis has been observed [100,114]. For example, the limited capacity of the adult human heart to spontaneously repair its damaged muscle following myocardial infarction (MI), an ischemia animal model, leads to loss of cardiac function, ventricular remodeling and progressive dysfunction frequently leading to heart failure. So prolonged heart ischemia caused by atherosclerosis or thromboemboli results in ischemic cardiomyopathy (IC) and myocardial infarction (MI), which may in turn elicit heart failure and death [115].

In clinic, despite improvements in emergency treatment, myocardial infarction often leads to congestive heart failure. Other than heart transplantation, current therapies are aimed at enabling the patient to survive with a heart working at a fraction of its original capacity. It is therefore no surprise that cardiac stem cell therapy has raised many hope, especially MSCs are better studies in animal models [116]. Unfortunately, neither the ideal source and type of stem cell nor the critical cell number and mode of application have been defined so far. If it is the case that NG2$^+$cells can be the source of MSCs that would cross a variety of faced obstacles in current clinical settings. A large gap in our understanding of what factors control NG2-expressing PCs differentiate into functional cells. In order to bridge this gap, we have purified NG2$^+$cells from adult mouse heart aortic arch (heart-NG2$^+$cells) by using the same procedure as CNS-NG2$^+$cells, eye (Iris)-NG2$^+$cells and BM-NG2$^+$cells and cultured them with myocardial infarction (MI) homogenate (Figure 4). Interestingly, we found that the heart-NG2$^+$cells turn on SMCs-expressing myofibronic progenitor cells when they present in MI homogenate.

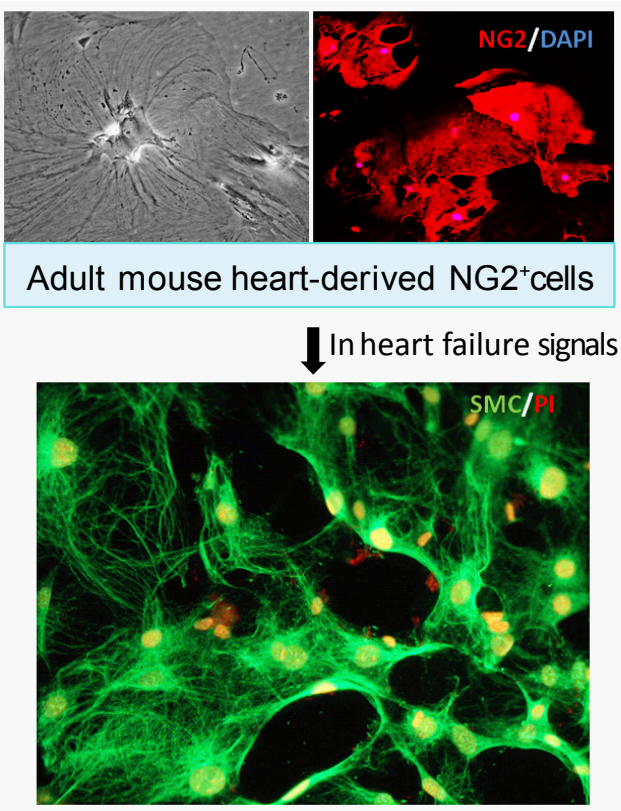

Figure 4: Mouse heart-derived NG2-expressing cells (heart-NG2$^+$cells) express myofibronic markers in response to insult signals. Mouse hearts from two C57Bl/6J mice (> 8 wks) were used to obtain NG2$^+$cells. The rest of procedures to isolate NG2$^+$cells were performed as described above. The prepared NG2$^+$cells were cultured in the presence of heart homogenates derived from atherosclerosis mice fed with high cholesterol food. Representative images show that mouse heart-derived NG2-expressing cells expressed myofibronic markers when cultured in the presence of heart homogenates derived from atherosclerosis mice fed with high cholesterol food (insult signals).

NG2$^+$cells in other multiple organs for tissue repair

Existed NG2$^+$cells have also been recognized in other adult organs including heart, liver, pancreas, lung and kidney where they maintain specialized functions [117], suggest these cells may act as contractile cells and obligatory regulators for vascular development, stabilization, maturation and tissue remodeling.

NG2-expressing cells in adult rodent liver share similar characteristics with PCs [99]. They express both PC (PDGFR-β, CD146) and MSC markers (CD105, CD72) but do not express EC, astrocyte (GFAP) and hematopoietic stem cell (CD34) markers. The NG2-expressing PCs also shared some cell phenotypic markers with the MSCs that isolated from other organs [118] and possess an intermediate phenotype between vascular SMCs and fibroblasts with a capacity to differentiate into a myofibroblast phenotype. Morphologically, PCs in adult mouse liver possess long processes embracing the abluminal endothelium wall in pre-capillary arterioles, capillaries, and post-capillary venues. Long cytoplasmic processes that extend along and encircle the endothelial tube ideally position these cells for paracrine signaling with ECs. These varied morphologies have been translated into diverse functions. Interestingly, anatomic characteristics of hepatic stellate cells (HSCs) have led to the distinction of HSCs as an adult liver-specific PCs [107,119]. This new concept is recapitulated by studies of PCs in other organs that identify the protein role and relevant mechanisms of this cell type for vascular development and function.

In adult mammalian liver, the complementary processes of angiogenesis (vascular growth from preexisting vessels) and vasculogenesis (*de novo* blood vessel development) regulate vascular development and homeostasis. Liver regenerative medicine for liver disease repair is much related to this regulation and focused majorly on three major areas: (1) Liver cirrhosis, to improve regeneration and reduce scarring by modulating the liver's own regenerative processes, (2) Immunity-mediated liver damage to down-regulate inflammatory immune response, and (3) to offer hepatic cell differentiation from transplanted stem/progenitor to supplement or replace hepatocyte function. There were several studies reported that NG2-expressing cells in blood vessels express NG2 and CD146 proteoglycan, suggesting PC progenitor potential [120,121] although these rely mostly on indirect evidence. Of clinical interest, the role of PC-like HSCs in hepatic angiogenesis and remodeling comes from work using a pre-clinical animal model (liver fibrosis or cirrhosis) for liver regeneration [122-125].

The development of advanced fibrosis presents three further animal models that also showed similarities with NG2-expressing PC behaviors. In the first, the development of fibrosis and its spontaneous resolution, there is a progressive activation of HSCs to become myofibroblast-like progenitor cells. In the second, a form of sinusoidal remodeling occurs in more advanced cirrhotic liver. These scars contain myofibroblasts, likely derived from activated HSCs. Intriguingly, if a comparison is drawn between armature scar within a cirrhotic liver and a less mature scar, the cells within the mature scar express markers more commonly associated with HSCs than myofibroblasts progenitor cells, such as glial fibrillary acidic protein and desmin in the absence of α-SMA [126]. Lastly, good evidence now exists in the models of advanced human and rodent fibrosis for bone marrow stem cell contribution to the myofibroblast progenitor and HSC population [127,128]. However, each of the aforementioned differentiation experiments was performed on unpurified PCs. Take together with those *in vivo* experiments may support to understand mechanisms by which HSCs operate as "contractile machinery" and relax in response to toxic agents [129-131]. Good evidence supports a contraction-based vasoconstrictive function of HSCs in the setting of cirrhosis but the role of HSCs as a regulator of vascular tone in normal liver is less established. These results demonstrated that modulation of vasoactive agents such as endothelin and carbon monoxide cause changes in sinusoidal diameter at locations where HSCs reside [132]. The process of sinusoidal remodeling could be distinct from the more characterized role of HSCs in the process of collagen deposition and fibrosis [133], suggesting that these vasoregulatory changes occur outside of the hepatic sinusoids [134,135]. In liver cirrhosis, partial cirrhotic reversal is highly related to the level of fibrotic resolution and the state of cirrhotic progress [136] and these primary phenomena raise the possibility of slowing the development of liver cirrhosis [137].

According to a range of studies described above about NG2$^+$cell stem/progenitor potential, we propose adult NG2$^+$cells that purified from multiple organs such as CNS-NG2$^+$cells, eye (Iris-NG2), BM-NG2$^+$cells, liver-NG2$^+$cells, heart-NG2$^+$cells, pancreas-NG2$^+$cells, lung-NG2$^+$cells and kidney-NG2$^+$cells may all share the similar biological characteristics. They express both PCs (PDGFR-β, CD146) and MSC markers (CD73CD105). They may have stem/progenitor potential in response to insult signals and involve in repair processes (Figure 5).

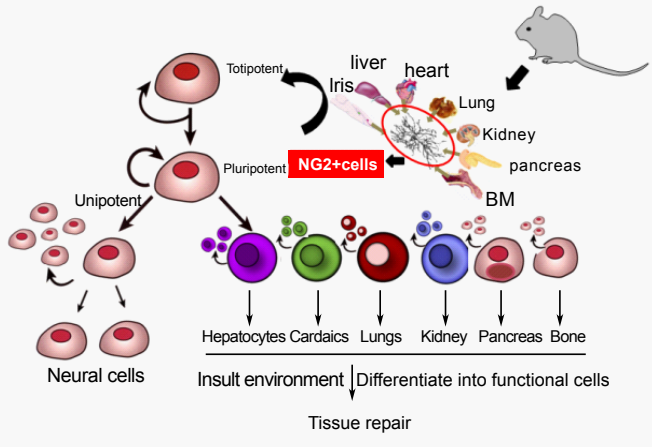

Figure 5: Hypothetic schematic presentation shows the stem/progenitor potential of NG2-expressing cells that purified from multiple organs and perform tissue repair in response to injury. In response to insult signals and perform repair, the multi-organ-derived NG2$^+$cells may differentiate into functional cells.

Unanswered Questions and Perspectives

Based on in the search for proof of resident NG2-expressing cells in the adult CNS, outside CNS organs and our preliminary studies demonstrate that the NG2$^+$cells would be a novel progenitor cells in regenerative medicine.

Much work has been done in the developmental system of the NG2$^+$cells, but many problems remain unsolved in the adult system. Although we have developed a novel approach for gleaning a sufficient number of NG2$^+$cell from adult multiple mouse organs, we still don't completely understand what is the potential role of these NG2-expressing stem/progenitors in adult mammalian organs. For example: (1) the possibility of tumorigenesis has not been tested. We propose, like other adult stem/progenitor cells such as MSCs, these adult NG2$^+$cells may be relatively safe compared to ESCs and iPSCs which readily form teratomas. (2) What is the nature of these adult NG2$^+$cells residing in the multiple organs? Are they "permanent resident" or just a "crossing guest" in blood vessel wall? We propose in normal condition, these adult NG2$^+$cells like traditional progenitors that has capacity to generate newly different cell types and reside "quietly" in blood vessel wall but can be activated in response to different insult semaphores not only injury alone to differentiate rapidly into repair cells. (3) Although a body of evidences provided by the review that these multiple adult organ-derived NG2$^+$cells share characteristics with PCs and MSCs, further research still need to be done on their different in unique niches, the contours underlying their genomics, proteomis and glycomics compare to traditional stem/progenitor cells, as well as the degree of immune rejection.

In sum, appeared adult NG2$^+$cells on the stage are the population that locates not only in the CNS as traditional thought but also outside CNS multiple organs such as eyes, bone marrow, heart, liver, lung, pancreas and kidney (multi-NG2$^+$cells). More analysis of multi-NG2$^+$cells will provide valuable insights into the cellular and molecular pathways that mediate recovery from multiple organ diseases. Such researches are fundamentally important for setting novel cell-based therapy in regenerative medicine.

Acknowledgements

This work was supported by a Grant from the Natural Science Foundation of China (No. 81170425. Special thanks to Shuai L, Lai J and Dr. Li Y for providing tissue samples and images organization.

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This article was originally published in a special issue, **Autoimmune Diseases** handled by Editor(s). Dr. Kenneth Blum, University of Florida, USA