● PERSPECTIVE

Potential utility of aldose reductase-deficient Schwann cells IKARS1 for the study of axonal degeneration and regeneration

Diabetic peripheral neuropathy (DPN) is one of the most common and intractable complications of diabetes mellitus. Its irritating symptoms, such as paresthesia, hyperalgesia and allodynia, can be causes of insomnia and depression; whereas its progression to more advanced stages can result in serious consequences, such as lower limb amputations and lethal arrhythmias. The pathogenesis of DPN remains largely unknown, but long-term exposure to hyperglycemia is likely to play a major role in metabolic and vascular abnormalities in the peripheral nervous system (PNS). In the PNS, blood glucose is transported into the cells in an insulin-independent manner. Under normoglycemic conditions, most of the cellular glucose is converted into pyruvate through the glycolytic pathway, and further metabolized in the cytosol or mitochondria. Under hyperglycemic conditions, however, saturation of the glycolytic pathway and augmentation of glucose flux into the several collateral pathways (*e.g.*, polyol pathway, hexosamine pathway, protein kinase C (PKC) pathway, and advanced glycation endproduct (AGE) pathway) appears to be detrimental to the PNS constituents, in particular, neurons, Schwann cells and blood vessels (Goncalves et al., 2017; Sango et al., 2017). Aldose reductase (AR), the first and rate-limiting enzyme in the polyol pathway, is predominantly localized to Schwann cells in the PNS. AR catalyzes the conversion of glucose to sorbitol using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor, and sorbitol dehydrogenase (SDH) catalyzes the conversion of sorbitol to fructose using nicotinamide adenine dinucleotide (NAD^+). The enhanced AR activity in Schwann cells under high glucose environments is thought to affect nerve functions through various mechanisms, *e.g.*, osmotic stress and impaired uptake of myo-inositol and taurine due to sorbitol accumulation, acceleration of AGE synthesis from fructose and its metabolites, and reduced activity of nitric oxide synthase (NOS) and glutathione reductase (GR) due to NADPH consumption by AR. Depletion of nitric oxide resulting from NOS inhibition can be a cause of diminished nerve blood flow, whereas a decrease in reduced glutathione (GSH) levels resulting from GR inhibition can trigger oxidative stress (Sango et al., 2014) (**Figure 1**). AR-deficient ($AR^{-/-}$) mice exhibited no obvious phenotypes in the PNS, and they were protected from diabetes-induced reduction of nerve conduction velocity (NCV) and GSH levels in sciatic nerves (Ho et al., 2000). In contrast, transgenic mice overexpressing human AR in Schwann cells displayed more advanced neurological manifestations than non-transgenic littermates under diabetic conditions (Song et al., 2003). These findings support the idea that AR hyperactivity is a major contributing factor in the development and progression of DPN.

We have established spontaneously immortalized Schwann cell lines from $AR^{+/+}$ and $AR^{-/-}$ C57BL/6 mice. These cell lines, designated as 1970C3 and IKARS1, respectively, showed spindle-shaped morphology with immunoreactivity to glial cell markers [*e.g.*, S100, glial fibrillary acidic protein (GFAP), and p75 low-affinity neurotrophin receptor ($p75^{NTR}$)] (Niimi et al., 2018). The absent AR expression in IKARS1 cells was confirmed by real-time reverse transcription (RT)-PCR, immunocytochemistry, and western blotting, whereas the deficient enzyme activity was verified by liquid chromatography coupled with tandem mass spectrometry analysis for the measurement of intracellular contents of sorbitol, fructose, and galactitol. In 1970C3 cells, exposure to high glucose (30 mM) for 24 hours tended to increase the contents of sorbitol and fructose, and exposure to galactose (25 mM) for 24 hours markedly escalated the galactitol contents. In IKARS1 cells, however, the same glucose and galactose insults failed to up-regulate the respective polyol contents. Furthermore, DNA microarray and subsequent real-time RT-PCR/western blot analyses revealed significant down-regulation of mRNA/protein expression for SDH and ketohexokinase (KHK), the enzymes downstream of AR in the polyol pathway, in IKARS1 cells relative to 1970C3 cells. These findings suggest that the polyol pathway is inactivated in IKARS1 cells under normal and hyperglycemic conditions. It is of interest to note that mRNA expression of AR-related enzymes, such as aldo-keto reductase (AKR) 1B7 and AKR1B8, and aldehyde dehydrogenases (ALDH1L2, ALDH5A1, and ALDH7A1), was significantly up-regulated in IKARS1 cells compared with 1970C3 cells. AR, a member of the AKR superfamily (murine AR is also known as AKR1B3), is involved in the detoxification of reactive biogenic aldehydes, such as methylglyoxal, 3-deoxyglucosone, and 4-hydroxynonenal (Sango et al., 2014). However, no significant differences in the viability between IKARS1 and 1970C3 cells after exposure to these aldehydes suggest that the aldehyde detoxification is taken over by the up-regulated AKRs and ALDHs in IKARS1 cells.

Impaired peripheral nerve regeneration after injury is a characteristic feature of DPN, and can be attributed to reduced synthesis/transport of neurotrophic factors, enhanced activity of the negative regulators of axonal regeneration (*e.g.*, phosphatase and tensin homolog deleted on chromosome 10 and Rho/Rho kinase), delayed Wallerian degeneration, and alterations of target tissues receptive

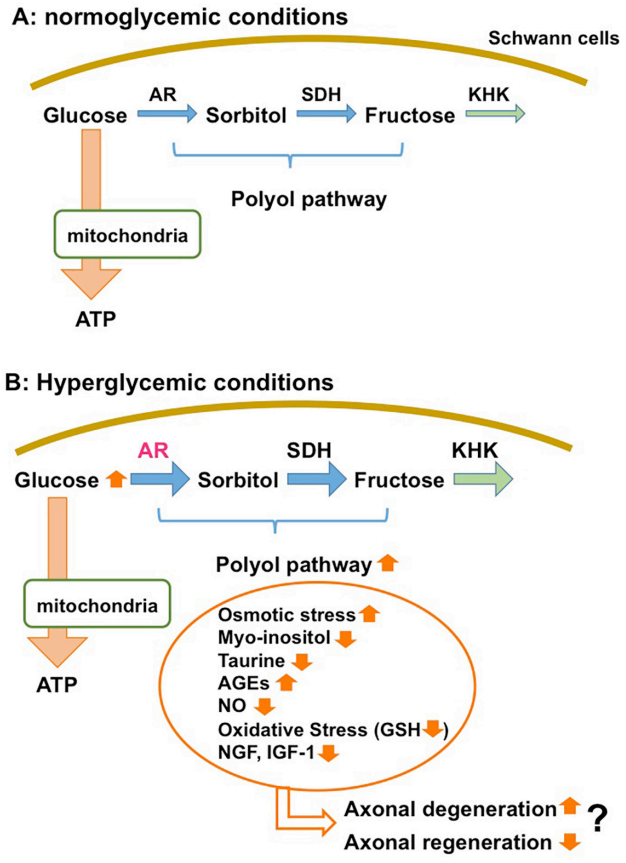

Figure 1 The polyol pathway hyperactivity in Schwann cells under hyperglycemic conditions can be involved in axonal degeneration and impaired axonal regeneration through various mechanisms.
AR: Aldose reductase; SDH: sorbitol dehydrogenase; KHK: ketohexokinase; AGE: advanced glycation endproduct; NO: nitric oxide; GSH: glutathione; NGF: nerve growth factor; IGF-1: insulin-like growth factor 1; ATP: adenosine triphosphate.

to reinnervation (Sango et al., 2017). Treatment with an AR inhibitor, epalrestat, preserved both NCV and nerve growth factor (NGF) levels of sciatic nerves in diabetic rats and secretion of NGF by primary cultured Schwann cells under a high glucose environment (Ohi et al., 1998). Similarly, epalrestat restored demyelinating changes and reduction of insulin-like growth factor 1 (IGF-1) levels in sciatic nerves of diabetic mice (Hao et al., 2015). These findings suggest that AR hyperactivity under diabetic conditions can be a cause of reduced synthesis and/or axonal transport of NGF/IGF-I, which in turn, may play a role in impaired axonal regeneration (**Figure 1**). In another study (Chen et al., 2010), diabetic $AR^{-/-}$ mice displayed improved Wallerian degeneration and axonal regeneration relative to that in diabetic $AR^{+/+}$ mice; AR deficiency resulted in the amelioration of retarded macrophage infiltration and impaired vascularization in the distal stump of the transected sciatic nerves under diabetic conditions. These findings agree with the restoring effects of AR inhibitors on the deficit of macrophage-associated process in Wallerian degeneration in diabetic rats (Sango et al., 2017). However, given the fact that AR is predominantly expressed in Schwann cells rather than macrophages in the PNS, it remains to be elucidated how the AR hyperactivity and increased glucose flux into the polyol pathway results in the impaired recruitment of macrophages. It would be interesting to explore the proliferative, migrating, and phagocytic activities of $AR^{+/+}$ and $AR^{-/-}$ Schwann cells during Wallerian degeneration under diabetic conditions. Tosaki et al. (2008) reported that conditioned media (CM) collected from a normal ICR mouse-derived Schwann cell line IMS32 under a high glucose condition (30 mM) exhibited less potent activity on the length and joint number of neurites emerging from adult mouse DRG neurons than that under a normoglycemic condition (5.5 mM). They also observed high glucose-induced reduction of NGF secretion by IMS32 cells. These findings, together with the ameliorating effects of epalrestat on the high glucose-induced reduction of NGF secretion by primary cultured Schwann cells (Ohi et al., 1998), led us to investigate if AR deficiency in Schwann cells alters the synthesis/secretion of neurotrophic factors. In our study (Niimi et al., 2018), CM collected from IKARS1 and 1970C3 cells increased the average neurite length and the survival ratios of adult rat DRG neurons, without significant differences in the bioactivities between the two cell lines. Further, the enzyme immunoassay revealed that both cell lines secreted NGF and glial cell line-derived neurotrophic factor (GDNF) into culture medium. Because no significant differences were detected between the cell lines in the amount of these molecules released into culture medium, AR deficiency does not seem to impair the ability of Schwann cells to produce neurotrophic and neuroprotective molecules. However, it remains to be determined if the hyperglycemic insults affect the secretion of these molecules by 1970C3 and IKARS1 cells.

In summary, both IKARS1 and 1970C3 cells possess characteristic features of Schwann cells, such as immunoreactivity to glial cell markers and synthesis/secretion of neurotrophic factors. We are currently investigating if these cell lines can myelinate neurites in co-cultures with adult mouse DRG neurons and NGF-primed PC12 cells. In IKARS1 cells, the polyol pathway is inactivated under exposure to high glucose and galactose, whereas the aldehyde detoxifying function of AR may be taken over by other AKRs and ALDHs. These cell lines will be useful tools for studying the physiological and pathological roles of AR in the PNS, especially the involvement of AR and the polyol pathway in axonal degeneration and regeneration under normal and diabetic conditions. However, it is important to bear in mind that the interactions among neurons, Schwann cells, and blood vessels are associated with the peripheral nerve events, and AR localized to vascular endothelial cells (Jiang et al., 2006) and macrophages (Chen et al., 2010) may also contribute to those events. Therefore, the findings from IKARS1 and 1970C3 cells should be interpreted in reference to the *in vivo* studies with AR inhibitors or $AR^{-/-}$ mice.

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