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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Reduced NGF in Gastric Endothelial Cells Is One of the Main Causes of Impaired Angiogenesis in Aging Gastric Mucosa

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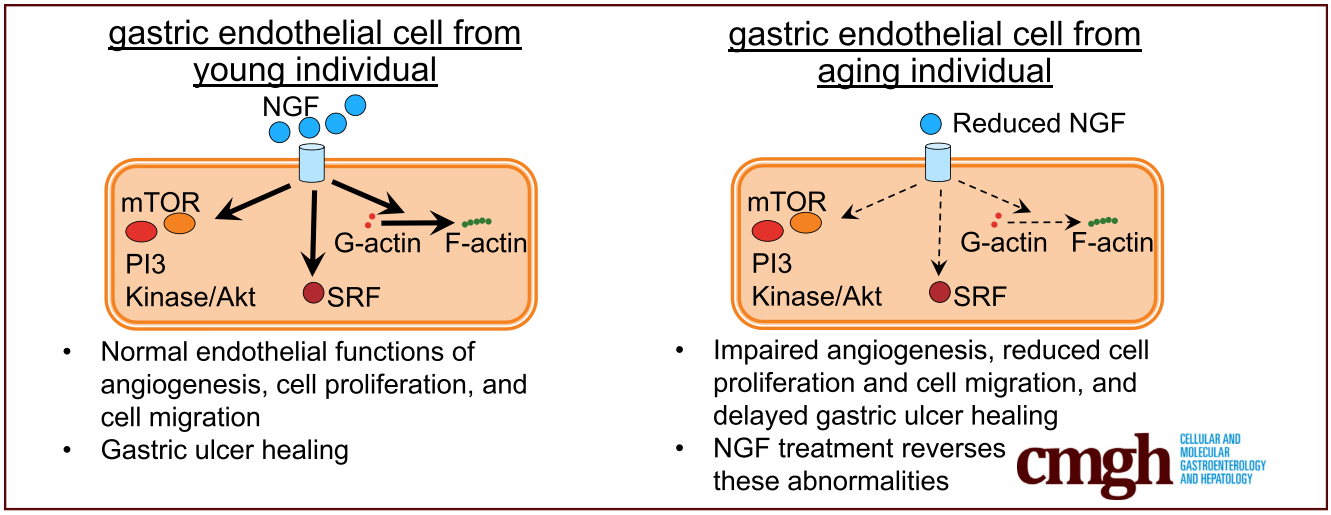

SUMMARY

This study detected reduced nerve growth factor (NGF) expression within gastric endothelial cells in both elderly patients and aged rats. Reduced NGF correlated with impaired angiogenesis and delayed gastric ulcer healing in aged rats. The defects could be reversed by exogenous NGF via phosphoinositide-3 kinase/serine threonine kinase signaling protein, and mammalian target of rapamycin signaling, and was dependent on serum response factor. These data show that down-regulation of endothelial NGF expression in aging is a significant contributor to impaired gastric mucosal repair.

BACKGROUND & AIMS: Aging gastric mucosa has increased susceptibility to injury and delayed healing owing to impaired angiogenesis, but the mechanisms are not fully known. We examined whether impairment of angiogenesis in aging gastric mucosa is caused by deficiency of nerve growth factor (NGF) in gastric endothelial cells (ECs), and whether NGF therapy could reverse this impairment.

METHODS: In gastric mucosal ECs (GECs) isolated from young and aging rats we examined the following: (1) in vitro angiogenesis, (2) NGF expression, and (3) the effect of NGF treatment on angiogenesis, GEC proliferation and migration, and dependence on serum response factor. In in vivo studies in young and aging rats, we examined NGF expression in gastric mucosa and the effect of NGF treatment on angiogenesis and gastric ulcer healing. To determine human relevance, we examined NGF expression in gastric mucosal biopsy specimens of aging (≥70 y) and young (≤40 y) individuals.

RESULTS: In cultured aging GECs, NGF expression and angiogenesis were reduced significantly by 3.0-fold and 4.1-fold vs young GECs. NGF therapy reversed impairment of angiogenesis in aging GECs, and serum response factor silencing completely abolished this response. In gastric mucosa of aging rats, NGF expression in GECs was reduced significantly vs young rats. In aging rats, local NGF treatment significantly increased angiogenesis and accelerated gastric ulcer healing. In aging human subjects, NGF expression in ECs of gastric mucosal vessels was 5.5-fold reduced vs young individuals.

CONCLUSIONS: NGF deficiency in ECs is a key mechanism underlying impaired angiogenesis and delayed ulcer healing in aging gastric mucosa. Local NGF therapy can reverse these impairments. *(Cell Mol Gastroenterol Hepatol 2018;6:199–213; https://doi.org/10.1016/j.jcmgh.2018.05.003)*

Keywords: Nerve Growth Factor; Angiogenesis; Endothelial Cells; Aging; Gene Therapy; Ulcer Healing.

Our previous studies have shown that the gastric mucosa of aging individuals, which we termed aging gastric mucosa or aging gastropathy,[1,2] has increased susceptibility to injury and delayed healing owing to impaired angiogenesis,[1,3–6] but the mechanisms are not fully elucidated. Angiogenesis (new blood vessel formation) is a fundamental process that is essential for reproduction, postnatal growth, and tissue injury healing.[7–13] Angiogenesis is impaired in aging tissues including aging gastric mucosa and results in inadequate revascularization and delayed injury healing.[3,14,15] Vascular endothelial growth factor (VEGF) A is a fundamental regulator of angiogenesis in general,[16–19] and in injured and ulcerated gastric mucosa.[3,12,13] Our previous studies have shown that angiogenesis in vivo and in vitro is reduced dramatically in the gastric mucosa of aging rats, and showed that reduced VEGF expression in endothelial cells (ECs) is one of the mechanisms.[3,4] Nevertheless, treatment with VEGF only partly reversed impaired angiogenesis in aging gastric ECs,[4] indicating an essential role for other factor(s) in addition to VEGF. Neither the identity of such a factor nor its mechanism of action has been fully explored.

Nerve growth factor (NGF), originally discovered by the 1986 Nobel Laureate Rita Levi-Montalcini[20,21] as a factor critical for growth and survival of neurons, has gained attention in recent years for its actions that extend beyond the promotion of neuronal survival and outgrowth.[22,23] One such non-canonical action of NGF is the ability to promote angiogenesis in brain capillary ECs.[24] The expression of NGF in aging gastric ECs and the mechanistic role of NGF deficiency in impaired angiogenesis of aging gastric ECs are not known.

Because gastric mucosal ECs (GECs) are the key cellular targets and effectors of gastric angiogenesis, this study aimed to determine whether reduced NGF expression in aging GECs is a primary cause of the impaired angiogenesis in aging gastric mucosa, and whether NGF therapy can reverse aging-related impairment of angiogenesis in vitro. We examined the expression of NGF in ECs isolated from gastric mucosa of young and aging rats, and the cellular and molecular mechanisms of NGF action on aging GECs, including the effect of NGF treatment on GEC migration, G-actin to F-actin polymerization, stress fiber formation, proliferation, and angiogenesis. We also examined whether signaling pathways phosphoinositide-3 (PI3) kinase/serine threonine kinase signaling protein (Akt) and mammalian target of rapamycin (mTOR) are involved and the requirement of serum response factor (SRF) for these NGF actions. In in vivo studies, we examined the expression of NGF in gastric mucosa of young and aging rats, and the effect of local treatment with NGF on in vivo angiogenesis, gastric ulcer healing, and mucosal regeneration in aging rats.

Here, we show that aging GECs have significantly reduced expression of NGF and reduced angiogenesis compared with young GECs, and that NGF deficiency is a key cause of impaired angiogenesis because NGF gene therapy completely reversed this impairment. Furthermore, our study showed that NGF's angiogenic action involves PI3 kinase/Akt and mTOR, encompasses G actin to F actin polymerization and stress fiber formation, and is critically dependent on SRF. Our in vivo studies in rats showed reduced NGF expression in ECs of blood vessels in both uninjured and ulcerated gastric mucosa of aging rats compared with young rats. In aging rats, local treatment of NGF at the base of gastric ulcers significantly increased mucosal blood flow and angiogenesis, accelerated ulcer healing, and improved mucosal regeneration. We also demonstrated the human relevance of our experimental findings by showing reduced NGF expression in gastric ECs in gastric mucosal biopsy specimens of aging (age, $\geq$70 y) vs young (age, $\leq$40 y) individuals.

Materials and Methods

Study Approval

All experimental studies in rats were approved by the institutional animal review committees: subcommittees for Animal Studies of the VA Long Beach Healthcare System (Long Beach, CA) and the Jagiellonian University Medical College (Krakow, Poland). Rats received humane care based on the National Institutes of Health recommendations outlined in the Guide for the Care and Use of Laboratory Animals. The use of archival human gastric mucosal biopsy specimens for immunostaining was approved by the Institutional Review Board of the Veterans Affairs Medical Center (Long Beach, CA). The human gastric biopsy specimens did not have any specific patient identifiers and the only inclusion criteria used were age, normal appearance on histology, and the lack of *Helicobacter pylori* and inflammation in mucosal biopsy specimens.

All authors had access to the study data and reviewed and approved the final manuscript.

Isolation of Gastric Mucosal ECs

GECs were isolated from Fisher F-344 rats (purchased from the National Institute on Aging, Bethesda, MD), 3 months of age (referred to as *young GECs*) and 24 months of age (referred to as *aging GECs*) using Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1/CD31) selection and magnetic bead separation as described previously.[4] ECs were identified by positive staining for Factor VIII–related antigen, CD31, and VEGF-R2, and by absence of staining

Abbreviations used in this paper: **Akt, serine threonine kinase signaling protein; BrdU, bromodeoxyuridine; EC, endothelial cell; FITC, fluorescein isothiocyanate; GEC, gastric mucosal microvascular endothelial cells isolated from rats; GU, gastric ulcer; LV-GFP, lentiviral green fluorescent protein; LV-NGF, lentiviral nerve growth factor; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; NGF, nerve growth factor; NSAID, nonsteroidal anti-inflammatory drug; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; PCR, polymerase chain reaction; PI3, phosphoinositide-3; siRNA, small interfering RNA; SRF, serum response factor; VEGF, vascular endothelial growth factor.**

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