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AntihypoxamiR functionalized gramicidin lipid nanoparticles rescue against ischemic memory improving cutaneous wound healing☆,☆☆

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Abstract

Peripheral vasculopathies cause severe wound hypoxia inducing the hypoxamiR miR-210. High level of miR-210, persisting in wound-edge tissue as ischemic memory, suppresses oxidative metabolism and inhibits cell proliferation necessary for healing. In wound-edge tissue of chronic wound patients, elevated miR-210 was tightly associated with inhibition of epidermal cell proliferation as evident by lowered Ki67 immunoreactivity. To inhibit miR-210 in murine ischemic wound-edge tissue, we report the formulation of antihypoxamiR functionalized gramicidin lipid nanoparticles (AFGLN). A single intradermal delivery of AFGLN encapsulating LNA-conjugated anti-hypoximiR-210 ($AFGLN_{miR\text{-}210}$) lowered miR-210 level in the ischemic wound-edge tissue. In *rep*TOP™*mito*IRE mice, $AFGLN_{miR\text{-}210}$ rescued keratinocyte proliferation as visualized by *in vivo* imaging system (IVIS). ^{31}P NMR studies showed elevated ATP content at the ischemic wound-edge tissue following $AFGLN_{miR\text{-}210}$ treatment indicating recovering bioenergetics necessary for healing. Consistently, $AFGLN_{miR\text{-}210}$ improved ischemic wound closure. The nanoparticle based approach reported herein is effective for miR-directed wound therapeutics warranting further translational development.

Key words: Ischemic wounds; Tissue oxygenation; miR-210; Keratinocytes proliferation; Lipid nanoparticles

Peripheral vasculopathies are primarily responsible for wound ischemia and hypoxia.[1] The biological response to ischemia depends largely on the state of hypoxia. If the hypoxia is moderate, allowing for sufficient residual oxygen to support tissue survival, cells minimize oxygen cost by economizing metabolism and make an effort to mount adaptive solutions. On the other hand, if the hypoxia is severe or near-anoxic, then adaptive rescue is no longer an option. Cells bring down oxygen cost to the threshold that separates survival and death. This is sustainable for a limited time within which the tissue may be rescued by intervention or it yields to necrotic death.[1]

Our previous work has recognized the induction of master hypoxamiR miR-210 in keratinocytes of ischemic wound-edge tissue.[2-4] Elevated miR-210 lowers oxygen cost of survival by repressing mitochondrial metabolism and attenuating keratinocyte proliferation. miR-210 also silences cell cycle proteins[2,3] (Figure S1). Such measures defend survival but oppose healing because tissue repair requires oxidative metabolism and cell proliferation. Although the state of oxygenation of the wound tissue may be corrected by intervention,[1,5,6] growth of wound tissue is limited by an abundance of miR-210 repressing mitochondrial metabolism and cell proliferation. Thus, miR-210 may be viewed as an "ischemic memory" at the wound-edge tissue that is in direct conflict with wound healing. Healing outcomes of such ischemic wound tissue may be maximized by sequestering miR-210. In the present work we report the development of a novel antihypoxamiR functionalized lipid nanoparticles (LNPs) that is able to rescue against the ischemic memory of miR-210 improving cutaneous wound healing.

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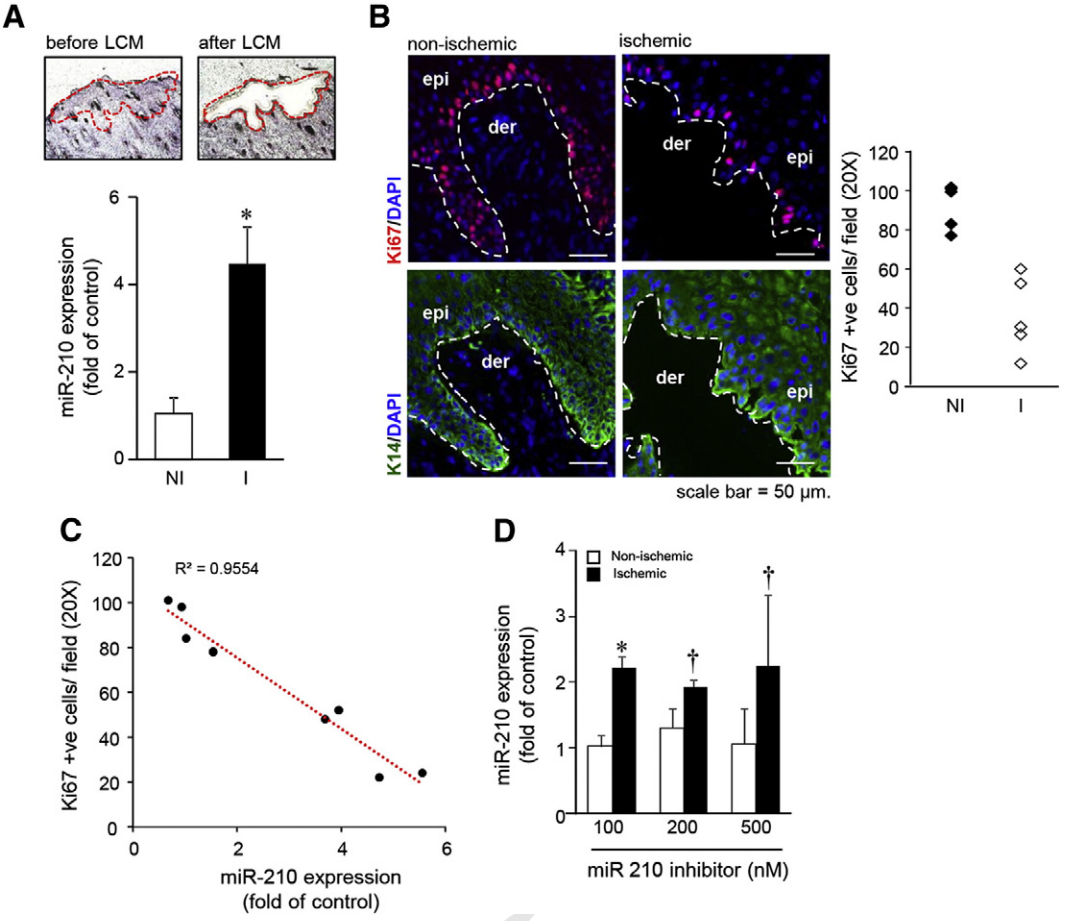

Figure 1. **(A)** *miR-210* expression from laser microdissected epidermis of human wound-edge tissue. (n = 7), * $P < 0.001$; ANOVA. **(B)** Serial human wound cross-sections stained with anti-Ki67 and keratin-14 antibody, counter stained with DAPI. (n = 4-5). The plot represents quantification of the Ki67 positive cells/ field (20X). **(C)** Regression plot of miR-210 expression from the human wound edge biopsies against number of Ki67 positive cells/field (20X). (n = 8) **(D)** *miR-210* expression from murine non-ischemic and ischemic wound-edge tissue 24 h after intradermal delivery of naked LNA-anti-miR-210. (n = 4). * $P < 0.01$; † $P < 0.05$ compared to non-ischemic wound, ANOVA.

Methods

1,2-Dioleoyl-3-dimethylammonium-propane (DODAP), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), soy phosphatidylcholine (SPC), gramicidin (GRAM), and d-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS) were dissolved in ethanol and combined at the molar ratio of 40/5/30/20/5 (DODAP/DOTAP/SPC/GRAM/TPGS). The lipid mixture was then combined with an appropriate amount of LNA based miR-210 power inhibitor in 40% ethanol followed by serial dilution (Appendix A. Supplementary data). Ischemic wound in C57BL/6 and *rep*TOP™*mito*IRE mice were induced by creating a bi-pedicle flap.[2] The *rep*TOP™*mito*IRE mice express the luciferase reporter gene under the control of an artificial minimal promoter derived from the Cyclin B2 gene, specifically induced during cell proliferation. LNA based anti-miR-210 and negative control were purchased from Exiqon. miRNA isolation, Western blot, and immunohistochemistry were performed as describe previously.[7] For further details see supplementary materials.

Results

Laser capture microdissection (LCM) of human ischemic wound-edge epithelium revealed elevated miR-210 expression compared to the non-ischemic epithelium (Figure 1, *A*). Expression of Ki67, a marker of cell proliferation, was also significantly lower in the epithelial tongue[8] of the human ischemic wound as compared to non-ischemic wound epithelium (Figure 1, *B*). The expression of miR-210 was inversely correlated with the number of proliferating keratinocytes in patients with chronic wounds (Figure 1, *C*). To evaluate the efficacy of anti-miR-210 in ischemic wound closure, a locked nucleic acid (LNA)-based anti-miR-210 power inhibitor (AM-210) was delivered intra-dermally to ischemic wound tissue of mice. At a relatively high dose of 500nM, administration of naked anti-miR-210 was ineffective in neutralizing hypoxia-induced elevated miR-210 in the wound tissue (Figure 1, *D*).

We sought to develop LNPs optimized for the delivery of antagomir cargo to the cutaneous wound tissue. The particle composition and zeta potential were evaluated and presented in