Aging-associated changes in oxidative stress, cell proliferation, and apoptosis are prevented in the prostate of transgenic rats overexpressing regucalcin

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Regucalcin (RGN) is a calcium (Ca^{2+})-binding protein that displays a characteristic downregulated expression with aging in several tissues. Besides its role in regulating intracellular Ca^{2+} homeostasis, RGN has been associated with the control of oxidative stress, cell proliferation, and apoptosis. Thus, the diminished expression of RGN with aging may contribute to the age-associated deterioration of cell function. In the present study, we hypothesized that the maintenance of high expression levels of RGN may prevent age-related alterations in the processes mentioned previously. First, we confirmed that RGN expression is significantly diminished in the prostate of 8-, 9-, 12-, and 24-months wild-type rats. Then, the effect of aging on lipid peroxidation, antioxidant defenses, cell proliferation, and apoptosis in the prostate of wild-type controls and transgenic rats overexpressing RGN (Tg-RGN) was investigated. The activity of glutathione and the antioxidant capacity were increased in Tg-RGN rats in response to the age-associated increase in thiobarbituric acid reactive substances levels, an effect not seen in wild type. Overexpression of RGN also counteracted the effect of aging increasing prostate cell proliferation. In contrast to wild-type animals, the prostate weight of Tg-RGN did not change with aging and was underpinned by the diminished expression of stem cell factor and c-kit, and increased expression of p53. In addition, aged Tg-RGN animals displayed increased expression (activity) of apoptosis regulators, therefore not showing the age-induced resistance to apoptosis observed in wild type. Altogether, these findings indicate the protective role of RGN against the development of age-related pathologies, such as, for example, prostate cancer. (Translational Research 2015;166:693–705)

Abbreviations: ABTS = 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate); Ca^{2+} = calcium; CypA = cyclophylin A; FasL = Fas ligand; FasR = Fas receptor; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; GST = glutathione S-transferase; MDA = malondialdehyde; pNA = p-nitroaniline; qPCR = Real-time PCR; RGN = regucalcin; ROS = reactive oxygen species; SCF = stem cell factor; SOD = superoxide dismutase; TAC = total antioxidant capacity; TBA = thiobarbituric acid; TBARS = thiobarbituric acid reactive substances; Tg-RGN = transgenic rats overexpressing RGN; WB = western blot

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AT A GLANCE COMMENTARY

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Background

Regucalcin (RGN) is a calcium-binding protein that is downregulated with aging. Moreover, RGN has been associated with the control of oxidative stress, cell proliferation, and apoptosis, which suggests that its diminished expression accompanying aging may contribute to the age-associated deterioration of cell function. However, whether maintaining RGN levels may prevent age-related alterations is unknown.

Translational Significance

Transgenic overexpression of RGN prevented aging-associated changes in oxidative stress, cell proliferation, and apoptosis in the rat prostate. These findings indicate that the modulation of RGN levels may have application to alleviate age deterioration of cell function and likely to prevent the progression of prostate cancer.

INTRODUCTION

Regucalcin (RGN) was first described in 1978 as a calcium (Ca^{2+})-binding protein, that does not contain the typical EF-hand Ca^{2+}-binding motif.[1] Approximately 2 decades later, it was identified as a senescence marker protein-30 because of the downregulated expression with aging in several rat tissues, namely, liver, kidney, and testis.[2,3] Despite the ability of RGN in regulating intracellular Ca^{2+} homeostasis,[4,5] different roles in cell physiology have been proposed for this protein in the last years. RGN seems to act as an antioxidant protein in rat liver, kidney, and brain by exerting a dual function, such as, inhibiting the activity of enzymes involved in the generation of reactive oxygen species (ROS) or enhancing the antioxidant defenses.[4] In addition, a role for RGN in the control of tissue cell homeostasis has been established. Our previous work demonstrated that RGN decreases cell proliferation and apoptosis in the rat prostate, concomitantly with altered expression of cell cycle (p21 and p53), and apoptosis (B-cell lymphoma 2 [Bcl-2], bcl-2-like protein 4 [Bax], caspase-8 and caspase-3) regulators.[6] On the other hand, the diminished expression of RGN has been observed in a set of human cancer cell lines and also in liver, breast, and prostate cancer tissues.[7-9] Altogether, the present data suggest that the diminished expression of RGN with aging may contribute to deregulated tissue physiology favoring the development of age-related diseases. Inversely, it is liable to assume that maintaining the expression levels of RGN may be a protective mechanism against age-related alterations in oxidative stress, cell proliferation, and apoptosis.

Given that aging is a major risk factor for prostatic diseases, the present study aimed to establish the expression pattern of RGN along the development and aging of the rat prostate. Furthermore, using transgenic rats overexpressing RGN (Tg-RGN), we determined the impact of maintaining high expression levels of RGN in preventing the age-associated changes in lipid peroxidation and antioxidant defenses, as well as in cell proliferation and apoptosis in the rat prostate.

MATERIALS AND METHODS

Animals and tissue collection. Sprague-Dawley wild type and Tg-RGN (n = 7 in each group) were obtained from Charles River (Barcelona, Spain) and Japan Salt Lake city (Hamamatsu, Japan), respectively. Tg-RGN was originally generated by Yamaguchi et al[10] by oocyte transgene pronuclear injection and has been used as a useful model to study the role of RGN in cell proliferation and apoptosis.[6,7,11] In addition, the overexpression of RGN in the prostate of Tg-RGN was previously demonstrated.[6]

Juvenile (20 days, 1- and 2-months [M]), young (3- and 4-M), middle-aged (6-, 8-, 9-, and 12-M), and aged (24-M) rats were housed under a 12-hour light, 12-hour dark cycle, with food and water available ad libitum, and handled in compliance with the National Institutes for Health guidelines and the European Union rules for the care and handling of laboratory animals (Directive 2010\63\EU). Animals were euthanized under anesthesia (Clorketam 1000; Vetoquinol, Lure, France), and whole prostates were removed, weighted, and frozen in liquid nitrogen for RNA and protein extraction. The number of animals in each age group was n = 7.

Real-time PCR (qPCR). Total RNA was extracted from rat prostates using TRI reagent (Sigma-Aldrich, St.Louis, Missouri) according to the manufacturer's instructions. After evaluating the quantity and integrity of total RNA,[6] 1 μg of RNA was reverse transcribed in a final volume of 20 μL using the First Strand complementary deoxyribonucleic acid (cDNA) Synthesis Kit (NZYTech, Lisboa, Portugal), following the manufacturer's instructions. One microliter of synthesized cDNA was used in each real-time polymerase chain reaction (qPCR) reaction carried out in the CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, California). The optimization strategy for each primer set, the specificity and