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Regucalcin: Molecular Function and Potential Roles in Cancer, Aging, Senescence, Frailty and Other Chronic Diseases

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Abstract: The calcium-binding protein known as regucalcin (RGN/SMP30), which lacks the EF-hand motif in its calcium-binding domain, was first identified in 1978. This calcium-binding protein with the potential to control the activation of numerous Ca^{2+}-dependent enzymes in liver cells was given the proposed name regucalcin. Then, it was discovered to be preferentially expressed in the liver, kidney and other tissues. The regucalcin gene is found on the X chromosome and numerous signaling factors regulate its expression. In various cell types, regucalcin is important for controlling intracellular calcium homeostasis. Regucalcin affects nuclear function, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis and has a suppressive effect on a number of signaling routes from the cytoplasm to the nucleus in proliferating cells. Regucalcin functions as a suppressor in the progression of several cancers in humans, including pancreatic, breast, hepatocellular and lung cancers. Studies in rats indicated that this protein may also play a role in aging, as it shows age-associated down-regulation. To determine the diagnostic, prognostic and therapeutic potential of frailty biomarkers, regucalcin was suggested as a core gene (protein). Moreover, regucalcin was reported to play a pathophysiological role in metabolic disorders. The expression of regucalcin is stimulated through the action of insulin in liver cells *in vitro* and *in vivo* and it is decreased in the liver of rats with type I diabetes induced by streptozotocin administration *in vivo*. This review will discuss the molecular function of regucalcin and its involvement as a suppressor protein in cancer, aging, senescence, frailty and other chronic diseases.

Key words: Regucalcin · Cancer · Aging · Chronic Diseases

INTRODUCTION

Regucalcin (RGN): Yamaguchi (1978) was the first to isolate regucalcin (RGN), a calcium-binding protein with a molecular weight of 33 kDa, from rat liver. It has been demonstrated that calcium homeostasis, signal transduction, nuclear gene expression, cell proliferation and apoptosis are all regulated by regucalcin in a variety of cells and tissues. Additionally, it possesses cytoprotective properties that lower intracellular oxidative stress levels [1, 2]. Regucalcin also has antioxidant and anti-inflammatory properties [3]. This protein exhibits age-associated down-regulation, which suggests that it may also contribute to aging, according to studies in rats. Senescence marker protein 30 (SMP30) is another name for RGN [4]. On chromosome X, this gene is a component of a gene cluster (Xp11.3-Xp11.23). Two transcript variants with different 5' UTRs encoding the same protein are produced as a result of alternative splicing [5].

Expression Patterns in Healthy Tissues/Organs: The maintenance of intracellular calcium homeostasis and liver metabolism relies greatly on RGN. Numerous enzymes, including pyruvate kinase, succinate kinase, glycogen phosphorylase and adenosine, are influenced by RGN [6]. Along with other tissues, liver, renal cortex, heart and brain, regucalcin has multiple functions in the control of cell functioning [6-10].

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The expression of the regucalcin gene can be increased by a number of transcriptional factors. Rat hearts were found to produce nuclear factor I-A1 (NFI-A1), a transcription factor and it was shown that NFI-A1 preferentially binds to the TTGGC pattern in the regucalcin gene promoter region [11] and was shown to specifically bind to the TTGGC motif in the regucalcin gene promoter region [11]. Rat heart expression of RGPR-p117, a novel transcription factor that binds to the regucalcin gene promoter region was also discovered [12].

The liver regulcalcin concentration was around 0.1 mM. The kidney also had a relatively higher level of regucalcin than the skeletal muscle, duodenum, testes, lung, heart, spleen, cerebral cortex and hippocampus. Regulacalcin was also prominent in the liver and barely noticeable in the kidney in female rats. As a consequence, the liver was the only tissue where regucalcin was specifically localized in rats. Rats' liver regucalcin concentration changed as they aged; it grew linearly in the first five weeks after male rats were born before progressively starting to decline [13].

The immunohistochemical investigation was initially used to demonstrate that regucalcin was expressed in rat hearts [6]. Regucalcin is present in the cytoplasm but not in the microsomes of rat heart cells and regucalcin mRNA is expressed in the muscle of the rat heart [10, 14]. Regucalcin levels in heart muscle tissues were estimated to be about 3.86×10^{-8}M [10].

In a variety of tissues, regucalcin generally seems to be a strong protective molecule against oxidative stress and chronic inflammation [15].

Molecular Functions: Due to the activation of Ca^{2+} pump enzymes in the plasma membrane (basolateral membrane), microsomes (endoplasmic reticulum) and mitochondria of many cell types, regucalcin plays a crucial part in maintaining intracellular Ca^{2+} homeostasis. Regucalcin inhibits proliferating cells' ability to transmit Ca^{2+} signals from the cytoplasm to the nucleus. Additionally, it has been demonstrated that the nuclear protein kinase, protein phosphatase, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis are all inhibited by the regucalcin, which can be transported to the nucleus. Regucalcin has the ability to control the increased cell proliferation brought on by hormonal stimulation [16].

It has been demonstrated that regucalcin plays a role in the regulation of cell nuclear function [17]. Importantly, it has been shown that regucalcin overexpression increases the expression levels of *p53* and *Rb*, tumor suppressor genes, as well as *p21*, a cell cycle inhibitor while suppressing the expression levels of *ras*, *c-jun* and *c-myc*, oncogenes, as a result of binding to nuclear DNA *in vitro* cloned H4-II-E rat hepatoma cells [17, 18].

Relationships with Aging, Senescence, Frailty and Telomere Function: In cells from diverse types of tissues, regucalcin has been shown to inhibit proliferation [19] and apoptotic cell death [20]. Regucalcin can therefore be essential for maintaining cell homeostasis [16].

Regucalcin, also known as senescence marker protein 30 (SMP30), is a novel class of Ca^{2+}binding proteins that lacks the EF-hand motif. The protein serves as a prominent aging marker that declines with age, as its names indicate [4]. By improving the plasma membrane's capacity to pump Ca^{2+}, the protein controls the homeostasis of intracellular calcium. They predicted the connection between SMP30 regulation and p53-mediated tumor suppression in a prior publication. High intracellular calcium is caused by age-related downregulation of SMP30 and is visible in senescent cells. Due to the fact that SMP30 has previously been shown to stimulate p53 induction [18], the age-mediated downregulation of SMP30 may partially limit p53-mediated Bax production under high intracellular Ca^{2+}, which would prevent apoptosis from being induced in response to any type of DNA damage [21].

Regucalcin also plays a pathophysiological role in diabetes and hyperlipidemia. Insulin activity in liver cells stimulates liver regucalcin gene expression, which is decreased in model animals with type I diabetes. Overexpression of regucalcin causes hyperlipidemia in rats by revealing hepatic insulin resistance and reduced liver triglyceride, total cholesterol and glycogen content. Regucalcin overexpression reduces the mRNA expression of liver leptin and adiponectin. In mice, regucalcin deficiency causes reduced glucose tolerance and lipid accumulation in the liver; in humans, it is linked to the onset and progression of nonalcoholic fatty liver disease and fibrosis. Regucalcin might be a crucial component in the lipid metabolic disease linked to diabetes and obesity [22].

However, SMP30 expression is downregulated during the aging process [21]; Its inhibitory effects have thus been observed to decrease with age in rats [23]. Nevertheless, despite the high potential of SMP30, only one study on the impact of exercise on SMP30 in humans

has been reported to date [24]. According to Pérez-Gómez *et al*., whole-body vibration exercise for 12 weeks increased postmenopausal women's SMP30 levels [24]. It is important to understand whether different exercise modalities influence SMP30 levels positively. It is conceivable to speculate that the effects of exercise may be mediated by the activity of SMP30 given that physical exercise and SMP30 appear to have synergistic effects on numerous markers of health and aging [25].

The involvement of regucalcin in aging and frailty has been further confirmed by studies in transgenic animals. SMP30 knockouts live shorter lives, have higher pro-inflammatory marker levels and develop Parkinson's disease and other age-related diseases at a higher rate [26]. Actually, vitamin C shortage causes decreased life spans and faster aging in SMP30 knockouts because regucalcin is essential for vitamin C production [27]. SMP30 overexpression, however, defends against age-related diseases [26, 28], a variety of insults brought on by stress, apoptosis and cell growth [29, 30]. Regucalcin levels drastically decrease with aging in tissues including heart, brain and prostate. These lower levels then cause cell senescence, frailty, fibrosis and other damage in organs like liver, heart, kidney and brain. Regucalcin is decreased in conditions such as fibrotic tissue in cirrhotic livers, various kidney diseases and heart failure. In these conditions, reduced regucalcin may play a significant pathophysiological role by impairing the activation of SOD (superoxide dismutase), an enzyme that in a healthy state prevents cell death and apoptosis in the heart. Reduced regucalcin levels have also been linked to worse survival across a variety of malignancies (including liver and pancreas cancer) [18, 31].

Expression Patterns in Cancer Diseases: Regucalcin expression has been demonstrated to be decreased *in vivo* in tumor tissues from mammalian and human subjects [32, 33]. Importantly, it has been demonstrated that regucalcin gene expression is decreased in a variety of human cancer tissues, suggesting that decreased regucalcin gene expression may promote the development of cancer [34]. The survival was prolonged in patients with pancreatic cancer [31], breast cancer [35], hepatocellular carcinoma [36] and lung cancer [37] who had tumor tissues with higher regucalcin expression compared to tumor tissues with lower regucalcin expression. These results are supported by the fact that regucalcin overexpression inhibited the development of human pancreatic cancer MIA The PaCa-2 cells [31], MDA-MB-231 breast cancer cells [35], liver cancer HepG2 cells [36], lung adenocarcinoma A549 cells [37], human colorectal carcinoma cells [38] and human renal cell carcinoma cells [39] *in vitro*. Regucalcin is important as a novel biomarker in the detection of human cancer since it may potentially suppress the development of carcinogenesis in human subjects. Prolonged survival in patients with colorectal cancer is associated with higher regucalcin gene expression. These results lend credence to the idea that regucalcin suppresses human cancer cells and that the downregulation of its gene expression results in the initiation of carcinogenesis in a variety of human organs. Regucalcin may therefore be a new target molecule for the diagnosis and therapy of human cancer [39].

Expression Patterns in Other Aging Diseases (Chronic Diseases): RGN is also known as a senescence marker protein since it expresses less as people age (SMP30). RGN also inhibits the expression of oncogenes, stimulates the expression of tumor suppressor genes and reduces cell proliferation in hepatoma cell lines [40].

RGN controls cell survival and apoptosis and has cytoprotective properties that lower intracellular levels of oxidative stress. RGN transcripts and protein levels are regulated by multiple factors, as well as cases of cancer, neurological illnesses and reproductive disorders have been linked to altered expression patterns of this interesting protein. Furthermore, RGN is a serum-secreted protein whose levels have been linked to disease stages, strongly indicating the potential utility of this protein as a biomarker to track the development and progression of the disease [21].

The most common cause of end-stage renal disease and a major complication of diabetes mellitus is diabetic nephropathy (DN). Early on, DN develops silently and without any clinical symptoms. It, therefore, calls for the identification of new markers to aid in diagnosis and outcome prediction. Zooming in on the locations of pathophysiological alterations is possible with tissue proteomics. Exosomes extracted from urine show a significant reduction in the expression of the regucalcin protein in kidney tissue from DN. A novel technique that should be investigated for the early diagnosis and progression of the course of diabetic kidney disease is urinary exosomal regucalcin [41].

Fujita *et al*. first identified SMP 30 as a senescence marker, with expression decreasing by as much as 40% in rat livers. Later research revealed that it was the same as regucalcin, a protein that controls intracellular Ca^{2+} homeostasis. Because SMP-30 has a secondary impact on vitamin C synthesis, its absence may also result in liver fibrosis. SMP30 has also been discovered to be significantly diminished in old zebrafish livers, as well as models of liver tumors and liver hepatectomy [42].

Diagnosis and Prognosis Values in Cancer Diseases: It has been demonstrated that the overexpression of regucalcin has inhibitory effects on the proliferation of human pancreatic cancer MIA cells PaCa-2 [31], MDA-MB-231 breast cancer cells [35], liver cancer HepG2 cells [36], lung adenocarcinoma A549 cells [37] and colorectal cancer RKO cells [38] *in vitro*. These results support the idea that regucalcin suppresses human cancer cells and that downregulating its gene expression causes carcinogenesis to occur in a variety of human organs. Therefore, regucalcin may be a new target molecule for the diagnosis and therapy of human cancer [39].

Regucalcin plays a physiological and pathological role in cell regulation and metabolic disorder in the kidney [43] and is expressed in proximal tubular epithelial cells of the rat kidney [10]. It should be mentioned that human kidney tumor tissues have down-regulated regucalcin gene expression and protein levels [34]. Regucalcin could therefore be a new target molecule for the diagnosis and therapy of renal cell cancer cells.

Before they progress locoregionally, or at distant sites as overt tumors, prostate cancers are known to be undetected or dormant for a long period of time. The molecular basis of dormancy is not yet fully known, though. A previous study conducted a differential gene expression analysis and discovered a gene called Regucalcin (RGN) that promotes the dormancy of prostate cancer. Patients with cancer who had higher levels of RGN expression had significantly longer overall and recurrence-free survival. The ectopic RGN expression in prostate tumor cells caused dormancy *in vivo*, but RGN suppression caused the recurrence of tumor growth using a doxycycline-inducible RGN expression system. On the other hand, silencing of RGN in LNCap cells endorsed its growth in the tibia of mice. Importantly, RGN supported several established indicators of tumor dormancy, including p38 MAPK activation, a reduction in Erk signaling and inhibition of FOXM1 expression. Additionally, RGN significantly reduced angiogenesis by raising the secretory level of miR-23c in exosomes. It's interesting to note that miR-23c expression in prostate cancer was discovered to be negatively regulated by FOXM1. In addition, 11 downstream RGN target genes were discovered. Importantly, RGN supported several established indicators of tumor dormancy, including p38 MAPK activation, a reduction in Erk signaling and inhibition of FOXM1 expression. Additionally, RGN significantly reduced angiogenesis by raising the secretory level of miR-23c in exosomes. It's interesting to note that miR-23c expression in prostate cancer was discovered to be negatively regulated by FOXM1. In addition, discovered 11 downstream RGN target genes that, on their own, indicated longer patient recurrence-free survival. We found that FOXM1 and/or p38 MAPK controlled the expression of these genes. These results point to a crucial role for RGN in the dormancy of prostate cancer and the usefulness of RGN signaling and exosomal miR-23c as biomarkers for recurrence prediction. that, on their own, indicated longer patient recurrence-free survival. FOXM1 and/or p38 MAPK were found to control the expression of these genes. These results point to a crucial role of RGN in the dormancy of prostate cancer and the usefulness of RGN signaling and exosomal miR-23c as biomarkers for recurrence prediction [44].

Possible Therapeutic Potential in Cancer: It has been demonstrated that the suppression of regucalcin gene expression is involved in carcinogenesis. Despite the expression of other genes being up-regulated, regucalcin gene expression was specifically down-regulated in rat liver carcinogenesis in vivo, suggesting that endogenous regucalcin suppresses the growth of hepatocarcinogenesis. Rat-cloned hepatoma cell proliferation was discovered to be suppressed in vitro by endogenous regucalcin overexpression. Furthermore, by analyzing numerous gene expression profiles and using proteomics, it was explicitly shown that the regucalcin gene and its protein levels were down-regulated in human hepatocellular carcinoma. Despite the expression of other genes being up-regulated, regucalcin gene expression was specifically down-regulated in rat liver carcinogenesis *in vivo*, suggesting that endogenous regucalcin suppresses the growth of hepatocarcinogenesis. Rat cloned hepatoma cell proliferation was discovered to be suppressed *in vitro* by endogenous regucalcin overexpression. Furthermore, by analyzing numerous gene expression profiles and using proteomics, it was explicitly shown that the regucalcin gene and its protein levels were down-regulated in human hepatocellular carcinoma. Regucalcin gene expression was also discovered to be suppressed in

human tumor tissues, such as the kidney, lung, brain, breast and prostate. This finding suggests that repressed regucalcin gene expression contributes to the emergence of carcinogenesis in a variety of tissues. Regucalcin might function as a suppressor protein in the development of cancer. It is hypothesized that endogenous regucalcin overexpression will reveal both therapeutic and preventative effects on carcinogenesis. Delivering the regucalcin gene could be a cutting-edge method for gene therapy of carcinogenesis [33].

One of the deadliest cancers is pancreatic ductal adenocarcinoma (PDAC), which is extremely aggressive and deadly. Due to pancreatic cancer's significant resistance to chemotherapy and radiotherapy, the clinical result is dismal. Increased regucalcin gene expression causes prolonged survival in PDAC patients. Additionally, it has been shown that overexpressing full-length regucalcin inhibits migration, cell death and proliferation in MIA cells from human pancreatic cancer (*K-ras*-mutated) that are resistant to chemotherapy and radiation. In MIA PaCa-2 cells, regucalcin may cause cell cycle arrest in the G1 and G2/M phases. Regucalcin's reduction of cell growth had no effect on cell death. Regucalcin overexpression was discovered to decrease *K-ras, c-fos* and *c-jun* oncogenes, by decreasing signaling pathways associated to K-ras signaling. It was also found to raise p53 protein levels, a tumor suppressor and suppress signaling pathways for Akt, MAP kinase and SAPK/JNK. The suppressor protein regucalcin may be involved in human pancreatic cancer [31].

In vitro colony formation and cell proliferation were inhibited by regucalcin overexpression in PC-3 and DU-145 bone metastatic human prostate cancer cells. Mechanically, overexpressed regucalcin lowered the levels of Ras, PI3 kinase, Akt and mitogen-activated protein kinase and increased the levels of p53, Rb and p21, suppressing cell proliferation. Additionally, higher levels of the transcription activity regulators nuclear factor-B p65, -catenin and signal transducer and activator of transcription 3 were reduced by higher amounts of regucalcin. Culture with the calcium agonist Bay K 8644 increased cell proliferation. Regucalcin overexpression prevented this effect. For example, when cocultured with preosteoblastic or preosteoclastic cells, overexpressed regucalcin inhibited the bone metastatic ability of PC-3 and DU-145 cells. Regucalcin can inhibit the growth of human prostate cancer, indicating that gene delivery systems that force its expression can be an innovative therapeutic approach [45].

REFERENCES

1. Singh, T., P. Banerjee, Uditi, S. Kumari, A. Chopra, N. Singh and I. Qamar, 2022. Expression of Regucalcin, a calcium-binding protein is regulated by hypoxia-inducible factor-1α. Life Sciences, 292: 120278.
2. Yamaguchi, M., 2014. Regucalcin, a novel regulatory protein implicated in obesity and diabetes. Integrative Molecular Medicine, 1: 7-10.
3. Jung, K.J., E.K. Lee, S.J. Kim, C.W. Song, N. Maruyama, A. Ishigami, N.D. Kim, D-S Im, B.P. Yu and H.Y. Chung, 2014. Anti-inflammatory activity of SMP30 modulates NF-κB through protein tyrosine kinase/phosphatase balance. Journal of Molecular Medicine (Berlin, Germany), 93: 343-56.
4. Jung, K.J., A. Ishigami, N. Maruyama, R. Takahashi, S. Goto, B.P. Yu and H.Y. Chung, 2004. Modulation of gene expression of SMP-30 by LPS and calorie restriction during aging process. Experimental Gerontology, 39: 1169-77.
5. Shimokawa, N., Y. Matsuda and M. Yamaguchi, 1995. Genomic cloning and chromosomal assignment of rat regucalcin gene. Molecular and Cellular Biochemistry, 151: 157-63.
6. Yamaguchi, M., M. Isogai, S. Kato and S. Mori, 1991. Immunohistochemical Demonstration of Calcium-Binding Protein Regucalcin in the Tissues of Rats: The protein Localizes in Liver and Brain. Chemical & Pharmaceutical Bulletin, 39: 1601-3.
7. Li, X., Y. Huang, S. Guo, M. Xie, X. Bin, M. Shi, A. Chen, S. Chen, F. Wu, Q. Hu and S. Zhou, 2019. Exogenous regucalcin negatively regulates the progression of cervical adenocarcinoma. Oncology Letters, 18: 609-16.
8. Misawa, H. and M.Yamaguchi, 2000. The gene of Ca2+-binding protein regucalcin is highly conserved in vertebrate species. International Journal of Molecular Medicine, 6: 191-7.
9. Shimokawa, N. and M. Yamaguchi, 1992. Calcium administration stimulates the expression of calcium-binding protein regucalcin mRNA in rat liver. FEBS Letters, 305: 151-4.
10. Yamaguchi, M. and M. Isogai, 1993. Tissue concentration of calcium-binding protein regucalcin in rats by enzyme-linked immunoadsorbent assay. Molecular and Cellular Biochemistry, 122: 65-8.

11. Misawa, H. and M. Yamaguchi, 2002. Identification of transcription factor in the promoter region of rat regucalcin gene: Binding of nuclear factor I-A1 to TTGGC motif. Journal of Cellular Biochemistry (84): 795-802.
12. Misawa, H. and M. Yamaguchi, 2001. Molecular cloning and sequencing of the cDNA coding for a novel regucalcin gene promoter region-related protein in rat, mouse and human liver. International Journal of Molecular Medicine, 8: 513-20.
13. Misawa, H., S. Inagaki and M. Yamaguchi, 2002. Suppression of cell proliferation and deoxyribonucleic acid synthesis in the cloned rat hepatoma H4-II-E cells overexpressing regucalcin. Journal of Cellular Biochemistry, 84: 143-9.
14. Yamaguchi, M. and R. Nakajima, 2002. Role of regucalcin as an activator of sarcoplasmic reticulum Ca2+-ATPase activity in rat heart muscle. Journal of Cellular Biochemistry, 86: 184-93.
15. Cardoso, A.L., A. Fernandes, J.A. Aguilar-Pimentel, M.H. de Angelis, J.R. Guedes, M.A. Brito, S. Ortolano, G. Pani, S. Athanasopoulou, E.S. Gonos, M. Schosserer, J. Grillari, P. Peterson, B.G. Tuna, S. Dogan, A. Meyer, Os. R. Van and A.U. Trendelenburg, 2018. Towards frailty biomarkers: Candidates from genes and pathways regulated in aging and age-related diseases. Ageing Research Reviews, 47: 214-77.
16. Yamaguchi, M., 2005. Role of regucalcin in maintaining cell homeostasis and function (Review). International Journal of Molecular Medicine, 15: 371-89.
17. Yamaguchi, M., 2011. Regucalcin and cell regulation: role as a suppressor protein in signal transduction. Molecular and Cellular Biochemistry, 353: 101-37.
18. Tsurusaki, Y. and M. Yamaguchi, 2004. Role of regucalcin in liver nuclear function: Binding of regucalcin to nuclear protein or DNA and modulation of tumor-related gene expression. International Journal of Molecular Medicine, 14: 277-81.
19. Yamaguchi, M., 2013. The anti-apoptotic effect of regucalcin is mediated through multisignaling pathways. Apoptosis, 18: 1145-53.
20. Yamaguchi, M., 2013. Suppressive role of regucalcin in protein output in liver cells. OA Biotechnology, 2: 6.
21. Marques, R., C.J. Maia, C. Vaz, S. Correia and S. Socorro 2013. The diverse roles of calcium-binding protein regucalcin in cell biology: from tissue expression and signalling to disease. Cellular and molecular life sciences: CMLS, 71: 93-111.
22. Yamaguchi, M., 2014. Involvement of Regucalcin in Lipid Metabolic Disorder and Diabetes. International Journal of Diabetes and Clinical Research, 1: 003e.
23. Yamaguchi, M., 2012. Role of regucalcin in brain calcium signaling: involvement in aging. Integrative Biology (Cambridge), (4): 825-37.
24. Pérez-Gómez, J., J.C. Adsuar, M.Á. García-Gordillo, P. Muñoz, L. Romo, M. Maynar, N. Gusi and R. PC, 2020. Twelve Weeks of Whole Body Vibration Training Improve Regucalcin, Body Composition and Physical Fitness in Postmenopausal Women: A Pilot Study. International Journal of Environmental Research and Public Health, 17: 3940.
25. Pérez-Gómez, J., P.C. Redondo, D. Navarrete-Villanueva, G. Lozano-Berges, I. Ara, M. González-Gross, J.A. Casajus and G. Vicente-Rodríguez, 2022. New Evidence on Regucalcin, Body Composition and Walking Ability Adaptations to Multicomponent Exercise Training in Functionally Limited and Frail Older Adults. International Journal of Environmental Research and Public Health, 19: 363.
26. Kim, Y.H., E.K. Lee, S.A. Park, N.H. Kim and C.W. Kim, 2012. Proteomic analysis of plasma from a Tau transgenic mouse. International Journal of Developmental Neuroscience, 30: 277-83.
27. Ishigami, A., 2010. Anti-aging Research Using SMP30/GNL Knockout Mice. Yakugaku Zasshi, 130: 25-8.
28. Vaz, C.V., D.B. Rodrigues, S. Socorro and C.J. Maia, 2015. Effect of extracellular calcium on regucalcin expression and cell viability in neoplastic and non-neoplastic human prostate cells. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1853: 2621-8.
29. Akhter, T., N. Sawada and M. Yamaguchi, 2006. Regucalcin increases Ca2+-ATPase activity in the heart mitochondria of normal and regucalcin transgenic rats. International Journal of Molecular Medicine, 18: 171-6.
30. Correia, S., C.V. Vaz, A.M.S. Silva, J.E. Cavaco and S. Socorro, 2017. Regucalcin counteracts tert-butyl hydroperoxide and cadmium-induced oxidative stress in rat testis. Journal of Applied Toxicology, 37: 159-66.
31. Yamaguchi, M., S. Osuka, M.N. Weitzmann, B.F. El-Rayes, M. Shoji and T.Murata, 2016. Prolonged survival in pancreatic cancer patients with increased regucalcin gene expression: Overexpression of regucalcin suppresses the proliferation in human pancreatic cancer MIA PaCa-2 cells *in vitro*. International Journal of Oncology, 48: 1955-64.

32. Yamaguchi, M., 2013. Suppressive role of regucalcin in liver cell proliferation: involvement in carcinogenesis. Cell Proliferation, 46: 243-53.
33. Yamaguchi, M., 2015. Involvement of regucalcin as a suppressor protein in human carcinogenesis: Insight into the gene therapy. Journal of Cancer Research and Clinical Oncology, 141: 1333-41.
34. Murata, T. and M. Yamaguchi, 2014. Alternatively spliced variants of the regucalcin gene in various human normal and tumor tissues. International Journal of Molecular Medicine, 34: 1141-6.
35. Yamaguchi, M., S. Osuka, M.N. Weitzmann, M. Shoji and T. Murata, 2016. Increased regucalcin gene expression extends survival in breast cancer patients: Overexpression of regucalcin suppresses the proliferation and metastatic bone activity in MDA-MB-231 human breast cancer cells *in vitro*. International Journal of Oncology, 49: 812-22.
36. Yamaguchi, M., S. Osuka, M.N. Weitzmann, B.F. El-Rayes, M. Shoji and T. Murata, 2016. Prolonged survival in hepatocarcinoma patients with increased regucalcin gene expression: HepG2 cell proliferation is suppressed by overexpression of regucalcin in vitro. International Journal of Oncology, 49: 1686-94.
37. Yamaguchi, M., S. Osuka, M. Shoji, M.N. Weitzmann and T. Murata, 2017. Survival of lung cancer patients is prolonged with higher regucalcin gene expression: suppressed proliferation of lung adenocarcinoma A549 cells in vitro. Molecular and Cellular Biochemistry, 430: 37-46.
38. Yamaguchi, M., S. Osuka and T. Murata, 2018. Prolonged survival of patients with colorectal cancer is associated with a higher regucalcin gene expression: Overexpression of regucalcin suppresses the growth of human colorectal carcinoma cells in vitro. International Journal of Oncology, 53: 1313-22.
39. Yamaguchi, M., S. Osuka, O. Hankinson and T. Murata, 2019. Prolonged survival of renal cancer patients is concomitant with a higher regucalcin gene expression in tumor tissues: Overexpression of regucalcin suppresses the growth of human renal cell carcinoma cells *in vitro*. International Journal of Oncology, 54: 188-98.
40. Vaz, C.A.V., 2010. Effect of Regucalcin on the Expression of Oncogenes and Tumor Suppressor Genes in Prostate Cell Lines. ProQuest Dissertations Publishing.
41. Zubiri, I., M. Posada-Ayala, A. Benito-Martin, A.S. Maroto, M. Martin-Lorenzo, P. Cannata-Ortiz, F. De La Cuesta, L. Gonzalez-Calero, M.G. Barderas, B. Fernandez-Fernandez, A. Ortiz, F. Vivanco and G. Alvarez-Llamas, 2015. Kidney tissue proteomics reveals regucalcin downregulation in response to diabetic nephropathy with reflection in urinary exosomes. Translational research: the Journal of Laboratory and Clinical Medicine, 166: 474-84.e4
42. Hodgson, R. and C. Christophi, 2015. What determines ageing of the transplanted liver? HPB, 17: 222-5.
43. Yamaguchi, M., 2015. The potential role of regucalcin in kidney cell regulation: Involvement in renal failure (Review). International Journal of Molecular Medicine, 36: 1191-9.
44. Sharma, S., X. Pei, F. Xing, S.Y. Wu, K. Wu, A. Tyagi, D. Zhao, R. Deshpande, M.G. Ruiz, R. Singh, F. Lyu and Watabe K. 2021. Regucalcin promotes dormancy of prostate cancer. Oncogene, 40: 1012-26.
45. Yamaguchi, M., S. Osuka, T. Murata and J.W. Ramos, 2021. Progression-free survival of prostate cancer patients is prolonged with a higher regucalcin expression in the tumor tissues: Overexpressed regucalcin suppresses the growth and bone activity in human prostate cancer cells. Translational Oncology, 14: 100955.