Review

EGR1 gene expression in Dietary Response

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Abstract

Various chemical components are daily taken into human body from dietary foods and supplements and initiate to change gene expression for homeostasis or adaptation. EGR1 (Early growth response protein 1) is a mammalian transcription factor encoded by EGR1 gene, which is induced by diverse signals. We propose that EGR1 can regulate an integrated action by food intake.

1. Introduction

Diet has a potential to change the human phenotype for adaptation during evolution as well as plasticity during homeostasis in a life. In lactase persistence associated with the ability to digest milk as adults, SNPs are associated with gene expression of lactase gene (LCT) and proved to enhance the transcriptional activity of LCT promoter in vitro.[1] The sensory genes in taste, olfactory, pheromonal and visual organs, which perform perception of chemicals, have been changed in DNA sequences with correlationship to diet. In a life, maternal and infancy diets can affect methylation status in epigenetic changes.[2] In contrast, there are conserved genes in evolution. Highly conserved proteins are often required for basic cellular function and therefore may be a conductor in changing human phenotype. One of these genes is EGR1, which acts as a transcription factor in gene expression of a network system for environmental stimuli and attributes in many biological functions. The gene expression of EGR1 is induced by food intake, pharmacological stimulations or physiological stimulations. Here, we review the induction of EGR1 in a network system of diet response.

2. General description of EGR1

EGR1 was first discovered by differential cloning from rat PC12 cells with nerve growth factor.[3] Subsequently, independent clones named as TIS8, EGR1 (EGR-1), Krox-24, and Zif268 are identified as each other. TIS8 is identified from tetradecanoyl phorbol acetate (TPA)-stimulated murine Swiss 3T3 cells by Lim et al.[4,5] EGR1 is identified by growth factor stimulation of quiescent fibroblasts in human and mouse by Sukhatme et al.[6] Krox-24 (Kruppel box) is identified from mouse NIH 3T3 cells stimulated to undergo the G0/G1 transition by serum by Lemaire et al.[7] Zif268 (zinc finger clone 268) is identified from stimulated BALB/c3T3 cells of mouse by Christy et al.[8] Other EGR1 homologs have been cloned and characterized from canary[9] and zebrafish.[10] The comparisons of EGR1 gene for phylogenetic diversity show that it is highly conserved. Conserved genes often play a fundamental role in basic life processes.

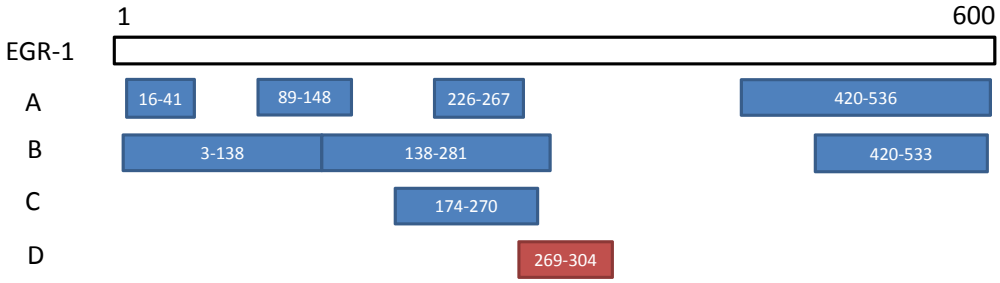

Fig1. The domain structure of EGR1 protein. The EGR1 of 1-600 amino acids is indicated in this figure (white bar). The activation domains (blue) and repression domain (red) of EGR1 protein is in A-D.
A) The indicated activation domains between amino acids 16-41, 89-148, 420-536, and 226-267 by Russo et al.
B) The indicated activation domains between amino acids 3-138, 138-281, and 420-533 by Gashler et al.
C) The indicated activation domain between amino acids 174-270 by Carman and Monroe et al.
D) The indicated repression domain between amino acids 269-304 by Russo et al.

EGR1 belongs to an "immediate-early response protein" because it is rapidly and transiently induced by growth factors, cytokines, differentiation signals, and DNA damaging agents. EGR1 can be induced in response to various stimuli, including memory[11,12], seizure[13], kindling[14], injury[15-17], apoptosis, oxygen deprivation[18,19], oxidative stress[20-22], various stress[23], atherosclerosis[24], cigarette smoke[25-28], ionizing radiation, and drug addiction and withdrawal. EGR1 is down-regulated during neurodegeneration.[29] The example of drug dependency is naloxone-precipitated withdrawal from morphine dependence[30], morphine withdrawal[30], ethanol withdrawal[31], acute amphetamine injection[32], and youkongdon injection.[33]

Human EGR1 encoded by EGR1 gene contains 543 amino acid residues. EGR1 binds the GC-rich sequence GCG (G/T) GGGCG, which often overlaps the DNA binding sequence of SP1. The alternative binding to EGR1 or SP1, which can be displaced, controls an inducible or basal transcription, respectively. The domains of EGR1 have been investigated to functionalize transcriptional activity in detail. The domain structure of EGR1 protein is depicted in Fig. 1. EGR1 contains three or four regions which are separated in the ability to stimulate transcription by gene manipulation: amino acids 16-41, 89-148, 420-536 and 226-267[34] or amino acids 3-138, 138-281, and 420-533.[35] These regions have an activation domain between amino acids 174 and 270[36], and a transcriptional repression domain between amino acids 269 and 304.[34] The repression domain is rich in basic amino acids which are an interface for interaction with other proteins.[36]

DNA-binding domain of EGR1 is analyzed by several crystal structures. EGR1 contains three tandem C2H2 zinc finger motifs.[8] The zinc fingers of EGR1 alone are sufficient for DNA-binding activity.[35,37] The zinc finger motif of 89 amino acid residues revealed the protein-DNA complex structure resolved to 2.1 Å by X-ray diffraction crystallography[37], shown in Fig. 2. The three zinc fingers are arranged in a semicircular structure that forced into the major groove of B-DNA. Each zinc finger domain consists of an antiparallel β-sheet and an α-helix folded by a zinc ion coordinated with two cysteine residues in the β-sheet and two histidine residues in the α-helix. Each finger can interact with a three base pair unit. First finger contacts the site

Fig2. Crystal structure of EGR1-DNA complex at 2.1 Å. The protein of EGR1 cDNA codes for the three zinc fingers. Each zinc finger domain consists of an antiparallel β-sheet (yellow) and an α-helix (red). Binding DNA is indicated by white

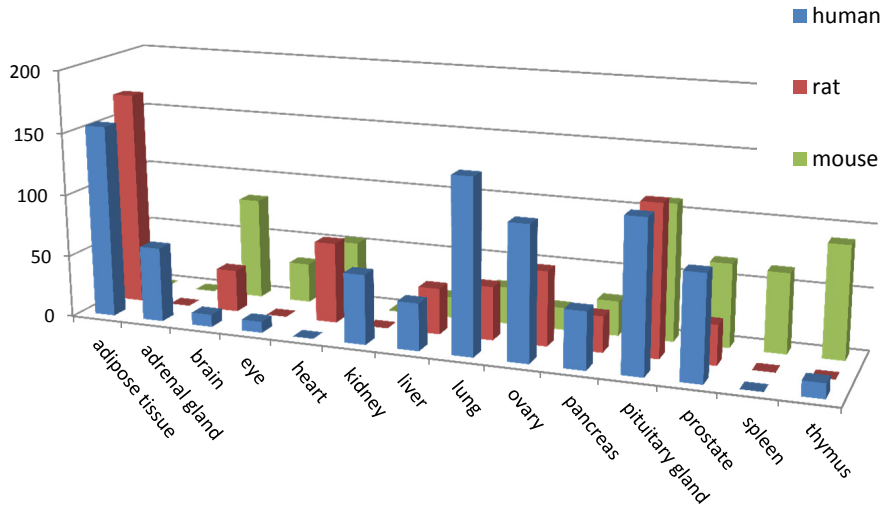

Fig3. EGR1 expression levels in various tissues of human (blue), rat (red), and mouse (green).

gcggggGCG, second finger contacts gcgGGGgcg, and third finger does GCGggggcg.

In tissue distribution, EGR1 expression levels of expressed sequence tag (EST) sequences from UniGene databases of human, rat, and mouse are shown in Fig. 3. EGR1 gene expression may not be tissue-specific but stimuli-dependent manner because EGR1 is expressed in various tissues. EGR1 gene is expressed at relatively high levels in the pituitary gland among them all. This is consistent with pituitary function in which endocrine hormones are secreted through hypothalamus by an integration of various stimuli. Therefore, the expression of EGR1 gene can be used for identifying target tissues at the period of environmental stimuli.

3. Induction of EGR1

3-1. Basal expression of EGR1

EGR1 mRNA and protein are induced after pharmacological stimulations or physiological stimulations. It is known that growth factor induces EGR1 protein.[38] Nervous system stimulation induces EGR1 mRNA and protein expression in brain. EGR1 mRNA was detectable as a 3.3kb transcript in both brain and lung of untreated SD rat.[3,39] The expression in the brain has been investigated in detail sections. EGR1 mRNA expression was detected in the mouse cortex and hippocampus by in situ hybridization.[8] Other study shows basal EGR1 mRNA expression in the rat neocortex primary olfactory and enthorhinal cortices, amygdaloid nuclei, nucleus accumbens, striatum, cerebellar cortex, and hippocampus.[40] EGR1 protein is highly expressed in rat neurons of cerebral cortex, hippocampus, thalamus, and striatum.[41,42,43] EGR1 is not detected in embryonic brain. These findings indicate that the basal expression of EGR1 is responsible to physiological stimuli involved neurotransmitters.

3-2. Inducible expression of EGR1 by neurotransmitter and its agonist/antagonist

Glutamate[44], N-methyl-D-aspartate (NMDA)[45], neurotoxins (quinolinic acid[46] and kainic acid[48]), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) / Quis agonists[44], dizocilpine[49,50], γ-Amino butyric acid (GABA) antagonist[50,51] (pentylenetetrazole, picotoxin and bicuculline), dopamine antagonist agonist[52-56], drugs (amphetamine[32] and cocaine[32,57]), adrenoceptor antagonist[58], muscarinc antagonist[55], morphine withdrawal[54,59], and vasointestinal peptide[53] are reported to induce EGR1 mRNA and protein in brain. Physiological stimulation and stress induce EGR1 mRNA and protein. (Table 1.)

3-3. Inducible expression of EGR1 by stress

Stimulation of touch[60] and light[61], maze training[62], restraint stress[58,63,64], noxious visceral[65], lithium chloride stress[66], formalin injection[67], sciatic nerve stimulation[68], axotomy[69], injury of brain[70-73], focal ischemia[74], electric shock[50,75], kindling[76], KCl[77], long-term potentiation (LTP)[50], oxygen deprivation[18,19], oxidative stress[20-22], heat shock, sodium arsenite, ultraviolet (U.V.) radiation, and anisomycin[23], shear stress[78,79], atherosclerosis[24],

Table 1. Regulation of EGR1 mRNA and/or protein expression

Up-regulation of EGR1 mRNA and/or protein expression by physical stimulation and stress			
Stimulus	Detection	Tissue	Literature
Glutamate	mRNA	Cortico-striatal monolayers	44
N-methyl-D-aspartate (NMDA)	mRNA, protein	Cerebral cortex, hippocampus, dentate gyrus, inferior colliculus, cerebellum, hypothalamus, olfactory bulb	45
Quinolinic acid	mRNA	Cerebral cortex, hippocampus, dentate gyrus	46
Kainic acid	mRNA, protein	Cerebral cortex, hippocampus, dentate gyrus	47, 78
Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)	mRNA	Neocortex, dentate gyrus, striatum, pyriform, cingulate and primary olfactory cortices, amygdala, lateral fornix, sensory, pyriform, and entorhinal cortces, dentate gyrus	44
Dizocilpine	protein	Retrosplenal and cingulate cortices, paraventricular and dorsomedial thalmic nuclei	49, 50
Pentylenetetrazole	mRNA, protein	Neocortex, pyriform and cingulate cortices, hippocampus, dentate gyrus	50, 51
Picotoxin	mRNA	Neocortex, pyriform and cingulate cortices, hippocampus, dentate gyrus	50, 51
Bicuculline	mRNA, protein	Entorhinal and cingulate cortices, amygdaloid and habenular nuclei	50, 51
Dopamine (D1) agonist	mRNA	Striatal neurons, cerebral cortical monolayers, caudate putamen, olfactory tubercle, cortex	52-54
Dopamine (D2) agonist	mRNA, protein	Caudate putamen, nucleus accumbens	54
Amphetamine (D1)	mRNA	Caudate putamen, nucleus accumbens, olfactory tubercle	32
Cocain (D1)	mRNA	Caudate putamen, nucleus accumbens	32
Cocain seizure	mRNA	Medial amygdala, striatum, ventral medial thalamus, pyriform and entorhinal cortices, hippocampus, dentrate gyrus	57
α 1 adrenoceptor antagonist	mRNA	Cerebral cortex	58
Muscarinc antagonist	mRNA, protein	Striatum	55
Morphine withdrawal	mRNA, protein	Cerebral cortex, hippocampus, dentate gyrus, cerebellum, brain stem, caudate putamen, nucleus accumbens, anterior cingulate cortex	54, 59
Vasointestinal peptide	mRNA	Cortical neurons	53
Tactile stimulation	protein	Somatosensory cortex	60
Light pulse	protein	Suprachiasmatic nucleus	61
Maze training	mRNA, protein	Brain	62
Restraint stress	mRNA	Cerebral cortex, hippocampus, thalamus, hypothalamus	58, 63, 64
Noxious visceral	protein	Lateral parabrachial area, venterolateral medulla oblongata, Edinger-Westphal nucleus	65
Lithium chloride stress	mRNA	Nucleus tractus solitaris, parabrachial nucleus, paraventricluar nucleus, central nucleus of amygdala	66
Formalin injection	protein	Parabrachial nucleus, medial thalamus	67
Sciatic nerve stimulation	protein	Parabrachial nucleus, doesal thalamic nuclei, lateral habanular nuclei, amygdala, lateral reticular nucleus, peroaqueductal gray, hypothalamic arcuate nucleus	68
Axotomy in central nervous system	protein	Induced in denervated areas	69
Focal cerebral injury	mRNA	Cerebral cortex, caudate putamen, pyriform cortex, paraventricular nucleus, dentate gyrus	70-72
Focal hippocampal injury	mRNA, protein	Dentate gyrus, non-nerve cells of pial surfaces, ventricles and surrounding the wound	73
Focal ischaemia	mRNA, protein	Induction in nerve and non-nerve cells depends on the ischaemia paradigm	74
Electric shock	mRNA, protein	Neocortex, pyriform and entorhinal cortices, dentate gyrus	50, 75
Kindling	mRNA	Neocortex, hippocampus, dentate gyrus, pyriform cortex	76
KCl	protein	Neocortex, pyriform and retosplenal cortices	77
Long-term potentiation (LTP)	mRNA, protein	Granule cells of the ipsilateral dentate gyrus	50
Oxygen deprivation	mRNA	Lung, osteosarcoma cell	18, 19
Oxidative stress	mRNA, protein	Osteoblastic cells, frontal cortex, striatum	20-22
Heat shock	mRNA, protein	NIH3T3 cell	23
Sodium arsenite	mRNA, protein	NIH3T3 cell	23
Ultraviolet radiation	mRNA, protein	NIH3T3 cell	23
Anisomycin	mRNA, protein	NIH3T3 cell	23
Shear stress	mRNA	Aortic endothelial cell	78, 79
Atherosclerosis	mRNA, protein	Atherosclerotic resion	24
Cigarette smoke	mRNA, protein	Lung	25-28
Ionizing radiateion	mRNA, protein	MCF-7 breast cancer cell	80
Stimulation of cannabinoid receptor	mRNA	Astracytoma cell	81
High-fat feeding	mRNA	Adipose tissue	82
Carrageenan	protein	Intestinal epithelial cell	83
Chronic ethanol	mRNA, protein	Kupffer cell, RAW 264.7 macrophages	86, 87
Fetal alcohol	mRNA	Brain	88
Caffeine	mRNA	Striatum	89
Capsaicin stress	mRNA	Cortex, striatum, paraventricular nucleus	90
Polycyclic aromatic hydrocarbons (PAHs)	mRNA	A549 lung carcinoma cell	91
Okadaic acid	mRNA	Myeloid Leukemin cell	92
Deoxynivalenol	mRNA	Hepatoma cell	94

Down-regulation of EGR1 mRNA and/or protein expression by physical stimulation and stress			
Stimulus	Detection	Tissue	Literature
Curcumin	mRNA	BKS-2 cell	96
Fructooligosaccharides	mRNA	Hroximal colon epithela	95
Zinc depletion	protein	Hep G2 cell	98

cigarette smoke[25-28], ionizing radiateion[80], and stimulation of cannabinoid receptor[81] are reported to induce EGR1 mRNA and protein in respective tissues. These results show that EGR1 may act as a master switch coordinating up-regulation of divergent gene families in stress conditions as well as physiological development.

4. EGR1 gene expression by foods or supplements

4-1. Up-regulation of EGR1 mRNA or protein expression

The expression of EGR1 has been reported to induce by high-fat feeding and food chemicals. EGR1 mRNA expression is increased in the white adipose tissue (WAT) after high-fat feeding. The up-regulation of EGR1 mRNA expression was demonstrated by Zhang et al.[82] They measured the expression level of EGR1 mRNA in the WAT of mice after high-fat feeding by quantitative PCR. They suggested it is closely associated with dietary-induced obesity and insulin resistance.

Carrageenan (CGN) is extracted from red seaweeds. It is composed of linear sulfated polysaccharides and widely used for gelling, thickening, and stabilizing in the food industry. CGN increases EGR1 protein expression in the intestinal epithelial cells. The up-regulation of EGR1 protein expression level was demonstrated by Choi et al.[83] They measured the protein expression level of EGR1 in human intestinal epithelial cells by Western blot analysis. They showed that EGR1 and other pro-inflammatory transcription factors were enhanced by CGN treatment.

Chronic ethanol exposure increases EGR1 mRNA and protein expression in the Kupffer cells and macrophages. Chronic ethanol exposure is known that it increases lipopolysaccaride (LPS), Kupffer cell activation, and TNF- α . It is reported that LPS increases the expression level of EGR1 protein and mRNA.[84-86] The up-regulation of EGR1 protein by LPS in Kupffer cells of rats feeding ethanol food was demonstrated by Kishore et al.[86] Chronic ethanol feeding increased the level of LPS-stimulated EGR1 gene expression.[86,87] Shi et al. showed that the EGR1 promoter activity in RAW 264.7 macrophages is increased by LPS stimulation.[87] They also demonstrated that increased EGR1 promoter activity and other (TNF- α mRNA accumulation) after chronic ethanol are prevented by overexpression of dominant negative ERK1/287. Prenatal exposure to ethanol produces fetal alcohol syndrome (FAS) in children. Fetal alcohol effect (FAE) increases EGR1 mRNA expression. The up-regulation of EGR1 mRNA was demonstrated by Nagahara et al.[88] They measured EGR1 mRNA level in brain regions of rat by in situ hybridization. They demonstrated that significant main effects of testing are observed for most brain regions analyzed, except for frontal area 1 and the dentate gyrus.

Caffeine is a xanthine alkaloid and a drug to act as a central nervous system stimulant. Caffeine is in coffee, tea, soft drinks, and chocolate derived from cocoa beans with varying quantities. Caffeine increases EGR1 mRNA in the striatum. The up-regulation of EGR1 mRNA was demonstrated by Svenningsson et al.[89] They analyzed the expression level of EGR1 mRNA in striatum of caffeine treated rats by in situ hybridization.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is an active component of chili peppers. In various Asian countries, it has traditionally been used for a basic taste as pungency. Capsaicin stress increases EGR1 mRNA in the hypothalamic paraventricular nucleus. Honkaniemi et al. analyzed the expression level of EGR1 mRNA by in situ hybridization to up-regulate EGR1 mRNA.[90] Stress induced by capsaicin injection increases EGR1 mRNA expression in the cortex, striatum and paraventricular nucleus.

Polycyclic aromatic hydrocarbons (PAHs) consist of fused aromatic rings such as naphthalene. They are potent atmospheric pollutants produced as byproducts of fuel burning and also found in cooked foods and cigarette smoking. PAH increases EGR1 mRNA expression in A543 lung adenocarcinoma cells. A number of PAHs have been known to affect several chronic diseases, including cancer and cardiovascular disease, in experimental animals following oral, pulmonary, dermal, or subcutaneous administration. The up-regulation of EGR1 by PAHs was demonstrated by Kim et al.[91] They measured EGR1 gene expression in A549 human lung adenocarcinoma cells treated PAHs (benz[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), chrysene (CHR), acenaphthene (ANY), anthracene (ANT), naphthalene (NAP), pyrene (PYR), phenanthrene (PA), and triphenylene (TP)) by transient transfection and luciferase reporter assays. They suggested that all the PAHs showed a significant increase in the gene expression of EGR1 except BaP and TP. Especially PA

and BaA were showed higher gene expression.

Okadaic acid (OA) is a cytotoxic polyether isolated from marine sponges of the genus Halichondria. OA causes diarrheal shellfish poisoning and increases the secretion of nerve growth factor.[92] The up-regulation of EGR1 mRNA was demonstrated by Kharbanda et al.[93] They studied EGR1 mRNA levels in Human myeloid leukemia cells by nuclear run-on assay.

Deoxynivalenol (DON) is a mycotoxin of the trichothecenes family that is mainly produced by Fusarium graminearum and F. culmorum. DON is commonly detected in cereals and grains, particularly in wheat, barley, and maize. The up-regulation of EGR1 by DON was reported by Nielsen et al.[94] They analyzed gene expression profiles by microarray and assayed by real-time PCR after exposing hepatoma cells to DON. They also quantified protein level of EGR1 of cells treated with DON by Western blot analysis. DON induced the expression of EGR1 in both levels of mRNA and protein.

4-2. Down-regulation of EGR1 mRNA or protein expression

Some of dietary fiber (DF) decreases EGR1 gene expression level in the proximal colon epithelia. Fructooligosaccharides (FOS) are oligosaccharides that occur naturally in plants such as onion, chicory, garlic, asparagus, banana, and wheat bran (WB). Down-regulation of EGR1 gene expression by FOS and WB were demonstrated by Chen et al.[95] They analyzed gene expression profiles that FOS and WB elicit in the proximal colon epithelia of rats fed with FOS diet by using microarray. They found that FOS and WB decrease the gene expression of EGR1.

Curcumin is a ginger family (Zingiberaceae) of the popular Indian spice turmeric. Han et al. demonstrated that curcumin decreases the expression of EGR1 mRNA in B cells.[96] They studied the expression of EGR1 mRNA of BKS-2 immature B cell lymphoma by Northern blot analysis.

There are reports that nutrition depletion down-regulates EGR1 protein expression. Previous studies have demonstrated that zinc plays important roles in cell proliferation.[97] Cui et al. measured EGR1 nuclear protein level in HepG2 cells by Western blot analysis and demonstrated that zinc depletion decreases EGR1 protein expression in the liver carcinoma cells.[98]

5. Induction of EGR1 expression by gut hormones

Gut hormones release during either feeding or fasting. Gut hormones modulate food intake. For example, Cholecystokinin (CCK) inhibits feeding[99] and ghrelin increases food intake.[100] Vagal afferent neurons (VAN) express receptors for many of the regulatory peptides and molecules released from the intestinal wall, pancreas, and adipocytes that regulate food intake and others. CCK interact with leptin in VAN[101] and increases the level of EGR1 in VAN. It was demonstrated by de Lartigue et al.[102] They determined the level of EGR1 gene expression in VAN by immunohistochemistry and Western blotting. CCK or leptin independently had little effect at lower doses, but leptin increases EGR1 expression in the presence of low doses of CCK. The dose-dependent response to CCK alone in VAN of diet induced obese (DIO) rats was identical to that of VAN from diet-induced obese resistant rats.[103] However, the ability of leptin to act synergistically with CCK in VAN of DIO rats was completely abolished.[102]

Ghrelin induces EGR1 protein in fasted rats. Hewson et al. demonstrated that systemic administration of ghrelin increases the number of cells expressing EGR1 protein in fasted rats and also that GHRP-6 (synthetic growth hormone secretagogue) increases the number of cells expressing EGR1 in fasted rats.[104] de Lartigue et al. demonstrated that ghrelin inhibits CCK-stimulated EGR1 and leptin-stimulated EGR1 expression.[103] They measured the level of EGR1 expression by immunohistochemistry. Ghrelin inhibited CCK8s (a synthetic octapeptide of CCK)-stimulated EGR1 redistribution to the nucleus.

6. Conclusions and future directions

The chemical information of food is integrated in the brain to control food intake, energy balance, and physiological actions[105] by signals from peripheral organs. The gastrointestinal tract, pancreas, and liver produce gastrointestinal hormones or peptides in response to various food stimuli and release them into the bloodstream for systemic effect, diffuse them as local messengers, or transmit them to the enteric nervous system to active nervous responses. The cooperative interaction of CCK, leptin, and ghrelin regulates EGR1 expression in vagal afferent neurons at gastrointestinal tract.[103] The vagal afferent neurons may be linked to activation of brainstem, hypothalamus

and motor cortex.[106] Systemic administration of ghrelin induces Fos and EGR1 proteins in the hypothalamic arcuate nucleus of fasted and fed rats.[104] Therefore, these suggest that the signal from peripheral organs induces the expression of EGR1 in the hypothalamus. On the other hand, some food can induce the gene expression of EGR1 in both brain and peripheral organs. The periphery increased expression of EGR1 is correlated with neuronal activity in response to food intake, which simultaneously occur the expression of EGR1 gene in the brain. EGR1 may be a potential marker to propose an axis of food (chemicals)-peripheral organs (sensing)-brain (behavior).

The expression of EGR1 gene in the brain is connected with brain function and social behavior[107] and finally influences survival and reproduction. Caffeine and capsaicin, which induce the expression of EGR1 gene in vivo, have been used in traditional medicine and may be useful for the linkage of EGR1 gene expression, brain function and social behavior.

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