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A Novel Divergent Gene Transcription Paradigm—the Decisive, Brain-Specific, Neural |-Srgap2–Fam72a-| Master Gene Paradigm

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Abstract

Brain development and repair largely depend on neural stem cells (NSCs). Here, we suggest that two genes, i.e., Srgap2 (SLIT-ROBO Rho GTPase–activating protein 2) and Fam72a (family with sequence similarity to 72, member A), constitute a single, NSC-specific, |-Srgap2–Fam72a-| master gene pair co-existing in reciprocal functional dependency. This gene pair has a dual, commonly used, intergenic region (IGR) promotor, which is a prerequisite in controlling human brain plasticity. We applied fluorescence cellular microscopy and fluorescence-activated cell sorting (FACS) to assess rat |-Srgap2–Fam72a-| master gene IGR promotor activity upon stimulation with two contrary growth factors: nerve growth factor (Ngf, a differentiation growth factor) and epidermal growth factor (Egf, a mitotic growth factor). We found that Ngf and Egf acted on the same IGR gene promotor element of the |-Srgap2–Fam72a-| master gene to mediate cell differentiation and proliferation, respectively. Ngf mediated Srgap2 expression and neuronal survival and differentiation while Egf activated Fam72a transcription and cell proliferation. Our data provide new insights into the specific regulation of the |-Srgap2–Fam72a-| master gene with its dual IGR promotor that controls two reverse-oriented functional-dependent genes located on opposite DNA strands. This structure represents a novel paradigm for controlling transcription of divergent genes in regulating NSC gene expression. This paradigm may allow for novel therapeutic approaches to restore or improve higher cognitive functions and cure cancers.

Keywords Brain · Cell cycle · Differentiation · Divergent transcription · Gene promotor · Proliferation

Abbreviations

Akt	AK strain transforming, AKT serine/threonine kinase
ANOVA	Analysis of variance
Atf1	Activating transcription factor 1
Bad	BCL2-associated agonist of cell death
Bak1	BCL2-antagonist/killer 1
Bax	BCL2-associated X
Bcl2	B cell lymphoma 2
Bcl2l1	Bcl2-like 1
Bid	BH3-interacting domain death agonist
Braf	B rapidly accelerated fibrosarcoma (B-Raf) proto-oncogene, serine/threonine kinase
BS	Binding site
BSA	Bovine serum albumin
Casp	Caspase
Ccna/b/d/e	Cyclin A/B/D/E
Cdk	Cyclin-dependent kinase
Cdkn1a	Cyclin-dependent kinase inhibitor 1A
Chr	Chromosome
CMV	Cytomegalovirus
CNS	Central nervous system
Creb1	cAMP-responsive element-binding protein 1
Cycs	Cytochrome c, somatic
DAPI	4′,6-Diamidino-2-phenylindole
DIC	Differential interference contrast
DMEM	Dulbecco's modified Eagle's medium
E	Exon
E2f	E2 transcription factor
EDTA	Ethylenediaminetetraacetic acid
Egf	Epidermal growth factor

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Egfr	Epidermal growth factor receptor
FACS	Fluorescence-activated cell sorting
Fam72a	Family with sequence similarity 72, member A
FBS	Fetal bovine serum
Fos	Finkel-Biskis-Jinkins (FBJ) murine osteosarcoma (Fos) proto-oncogene, activator protein 1 (AP-1) transcription factor subunit
GFP	Green fluorescent protein
Hdac1	Histone deacetylase 1
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HS	Horse serum
I	Intron
IGR	Intergenic region
IRES2	Internal ribosome entry site 2
KCLB	Korean cell line bank
Kmt5b/c	Lysine methyltransferase 5B/C
Map2k	Mitogen-activated protein kinase kinase
Mapk	Mitogen-activated protein kinase
Mcl1	Myeloid cell leukemia 1, BCL2 family apoptosis regulator
Mdm2	Murine double minute 2 proto-oncogene
MOMP	Mitochondrial outer membrane permeabilization
Mt1	Metallothionein 1
Myc	Myelocytomatosis viral oncogene
NCBI	National Center for Biotechnology Information
NDR	Nucleosome-depleted region
NEAA	Non-essential amino acids
Ngf	Nerve growth factor
NSC	Neural stem cell
Ntrk1	Neurotrophic receptor tyrosine kinase 1
P	Phosphorylation
P/S	Penicillin, streptomycin
pDNA	Plasmid DNA
Pik3cg	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma
PROMPT	Promoter upstream transcript
Rap1	Ras-related protein 1
Ras	Rat sarcoma (RAS) proto-oncogene, GTPase
Rb	Retinoblastoma (RB) transcriptional corepressor
RFP	Red fluorescent protein
SEM	Standard error of the mean
Srgap2	SLIT-ROBO Rho GTPase–activating protein 2
Suv39h1	Suppressor of variegation 3-9 homolog 1
TF	Transcription factor
TFBS	Transcription factor–binding sites
Tfdp1	Transcription factor dimerization partner 1 (Dp-1)
Tp53	Tumor protein 53
w/o	Without
+	Positive

Introduction

In the early 1940s, the modern age of molecular neurobiology started with research on growth and differentiation factors resulting in the 1986 Nobel Prize for physiology or medicine awarded to Rita Levi Montalcini and Stanley Cohen for the discovery of nerve growth factor (Ngf) and epidermal growth factor (Egf), respectively [1, 2]. Later, pioneering studies of cell cycle control by Leland H. Hartwell, R. Timothy Hunt, and Paul M. Nurse laid the foundation for our understanding of the mechanism of cancers, which is the basis of today's therapeutic strategies. In 2001, they were awarded the Nobel Prize for their discoveries of the molecular components and key regulators of the cell cycle and cell cycle checkpoints. The cell cycle is a complex, energy-demanding process with bidirectional signaling between cell division and general cell metabolism [3]. For multicellular organisms, developmental cell fate decisions are tightly regulated to allow spatial- and temporal-dependent cell differentiation [4]. During development of the central nervous system (CNS), cell cycle control is essential to coordinate the neuronal proliferation and differentiation required for brain plasticity [5, 6]. Moreover, in the adult brain, the mechanisms of neural stem cell (NSC) proliferation and consequently neurogenesis are of utmost significance for tissue regeneration [7].

The rat pheochromocytoma PC12 cell line is a well-established cellular model for characterizing cell proliferation, neuronal differentiation, and cell survival as well as neural regenerative and degenerative processes [8–13]. This cell line has been used to determine that mitogens and differentiation factors initiate multiple signaling pathways that converge on specific cellular targets to execute complex processes. These growth factors rely on canonical signaling cassettes and spatial and temporal integration of signal transduction events to define the different outcomes of Ngf and Egf, thereby inducing neuritogenesis and cell proliferation, respectively [8]. While a positive feedback loop in the mitogen-activated protein kinase (Mapk) network appears to be responsible for Ngf-mediated post-mitotic neuronal differentiation, a negative feedback loop upon Egf stimulation governs a proliferative cell fate [14–16].

The brain-specific neural |-Srgap2–Fam72a-| master gene constitutes a pivotal regulator of NSC proliferation and differentiation required for brain plasticity and synaptic connectivity, which allow for human cognition. More specifically, this |-Srgap2–Fam72a-| master gene contains two subgenes (Fig. 1) [17]: Srgap2 is involved in neuronal migration, neuronal

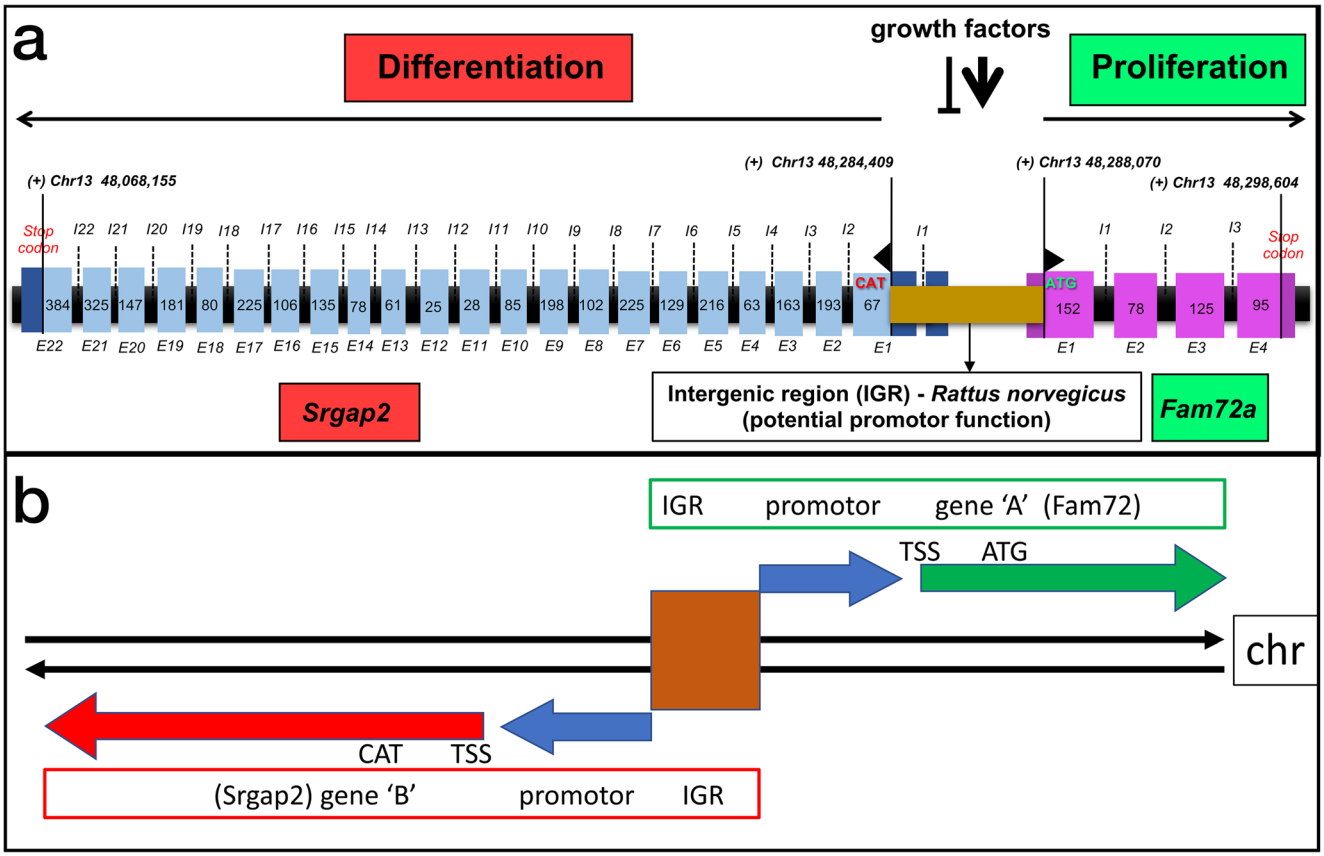


Fig. 1 The |-Srgap2–Fam72a-| master gene resides within a nucleosome-depleted region (NDR). The sequence was obtained from the National Center for Biotechnology Information (NCBI) database. Rat genomic Srgap2, rat genomic Fam72a, and the intergenic region (IGR) between these genes are indicated. **a** Rat Srgap2 and Fam72a are located and divergently expressed on opposite DNA strands on rat chromosome 13 (chr13 (chr1 in human)). Our early investigations used publicly available databases and describe potential transcription factor (TF)–binding sites (BS) (TFBS) within this IGR [17, 26]. For further details, refer to Kutzner et al. 2015 and Ho et al. 2017 [17, 26]. I, intron; E, exon; numbers inside the square boxes indicate the number of nucleotides for each exon. These data follow NCBI's Rnor_6.0 gene assembly. **b** Simplified divergent gene transcription scheme based on the PROMPT mechanism [32–34]. Reverse-oriented genes A and B are expressed from opposite DNA strands. TSS, transcription start site; ATG, translation start site. If gene A is activated by TFs, then, transcription of gene B is activated until it is actively terminated early [32]

differentiation, synaptogenesis, and neuritogenesis [18–23]; in contrast, Fam72a is a novel NSC self-renewal–supporting protein [24–26]. |-Srgap2–Fam72a-| master gene dysfunction could cause diseases such as cancer [17, 25, 27–30]. Thus, a full understanding of the specific transcription control mechanism of this master gene (Fig. 1a) may provide novel therapeutic approaches for neuroregeneration in dementia as well as cancer treatment.

The need to tightly control NSC proliferation and differentiation implies that Srgap2 and Fam72a share a dual promotor [26, 31]. A |-Srgap2–Fam72a-| master gene appears to be the most likely scenario providing a proof of concept for a new master gene control paradigm. Recent RNA sequencing data on the regulation of gene transcription indicate that gene promoters are largely divergent and initiate transcription of reverse-oriented promoter upstream transcripts (PROMPTs). These reverse-oriented promoters are positioned in separate, but closely spaced, nucleosome-depleted regions (NDRs). Under this paradigm, if transcription of gene "A" is initiated, transcription of gene "B," sitting on the opposite DNA strand, is incidentally initiated as well until transcription eventually terminates early [32]. Control of divergent gene transcription seems to occur across all eukaryotes though the functional relationship between such genes remains elusive (Fig. 1b) [33, 34].

In the current investigation, we analyzed the rat |-Srgap2–Fam72a-| master gene by assessing IGR promotor activity, divergent transcription regulation, and consequent cellular function and phenotype in rat PC12 cells and established a new divergent gene transcription paradigm.

Materials and Methods

Reagents

Unless indicated, all reagents were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Growth factors (recombinant rat Egf and rat Ngf) were purchased from PeproTech (Rocky

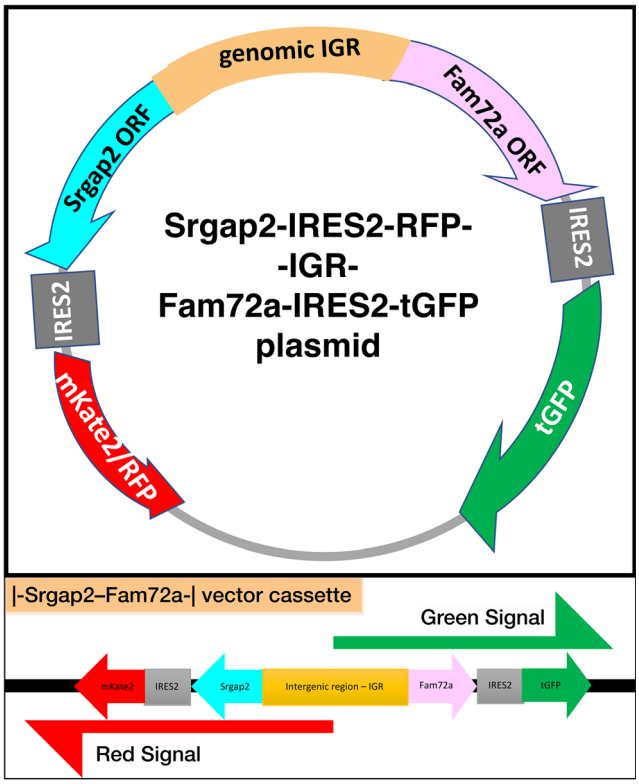


Fig. 2 A simplified map of the rat |-Srgap2–Fam72a-| master gene DNA plasmid including the genomic IGR sequence, Srgap2, Fam72a, and turbo green fluorescent protein (turbo GFP) as well as far-red fluorescent protein TagFP635 (mKate2/RFP) as reporter genes. Full rat genomic IGR DNA (~4kbp) and complete open reading frames (ORFs) of rat Srgap2 (3216 bp, XM_006249782.3) and rat Fam72a (450 bp, NM_001081451.1) were used. IRES2, internal ribosome entry site 2, an RNA element that allows for translation of fluorescent proteins in a cap-independent manner. The Srgap2 and Fam72a genes were only under control of the IGR promotor. Plasmid backbone: pGL3basic (Synbio Technologies, Monmouth Junction, NJ, USA)

Hill, NJ, USA).

Cell Culture

PC12 cells (Korean cell line bank (KCLB, No. 21721), Seoul, Rep. of Korea) were propagated in Dulbecco's modified Eagle's medium (DMEM containing 4.5 g/L glucose/F12 [1:1]) with 10% fetal bovine serum (FBS)/5% horse serum (HS) and 1% penicillin/streptomycin (P/S; 100 units/mL) (all from Gibco (Invitrogen), Carlsbad, CA, USA) on poly-L-ornithine hydrobromide/fibronectin (Sigma-Aldrich, St. Louis, MO, USA)–coated dishes (Corning, NY, USA) at 37 °C in humidified 5% CO_2/95% air [35].

PC12 cells were differentiated in the presence of Ngf (100 ng/mL) for 10–20 days [12].

Transfection of PC12 Cells

Low-passage-number PC12 cells (< 25 passages, < P25) were transfected with a Nucleofector™ device and Lonza Nucleofector™ cell kit under serum-free (without (w/o) serum) conditions according to the manufacturer's protocol (Lonza, Basel, Switzerland, #VPD-1001). Cells were transfected with a DNA plasmid containing reporter genes for green and red fluorescent proteins (GFP and RFP, respectively) under control of the |-Srgap2–Fam72a-| master gene to indicate IGR promotor activity (Fig. 2). For control experiments, standard DNA plasmids containing genes coding (under the control of a cytomegalovirus (CMV) promotor) only for GFP or RFP were used. Cells were plated on poly-L-ornithine hydrobromide/fibronectin–coated dishes. After transfection, cells were cultivated for 24 h in DMEM/F12 (1:1) media, and the medium was changed every other day. From day 2 after transfection, cells were treated with growth factors (Ngf or Egf) followed by fluorescence microscopy analysis for 10–20 days (Olympus IX71, Olympus Korea Co., Ltd., Seoul, Rep. of Korea; and Leica DM5000, Leica Microsystems Ltd., Seoul, Rep. of Korea) [11, 35].

Fluorescence-Activated Cell Sorting Analysis

At 10–20 days after stimulation with Ngf or Egf, induced PC12 cells were detached with trypsin (Gibco), collected by centrifugation, and resuspended in serum-free DMEM/F-12 (1:1) with 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Gibco) supplemented with 2% B27, 2 mM GlutaMax, non-essential amino acids (NEAA), and 1% P/S. To prevent cells from aggregating, 5 mM ethylenediaminetetraacetic acid (EDTA) and 0.5% bovine serum albumin (BSA) were added to the medium. Cells were strained through a 70-μm cell strainer (Falcon, Corning, NY, USA) and 1×10^7 cells/mL were subjected to fluorescence-activated cell sorting (FACS) analysis (FACSCanto™ II, BD Biosciences) according to the manufacturer's protocol. Obtained data were analyzed with FACS Diva software (BD Biosciences) and FlowJo (Tree Star, Ashland, OR, USA) [35].

Statistical Evaluation

Quantitative data obtained in this investigation are presented as mean ± standard error of the mean (SEM). Differences between groups were established using unpaired two-tailed *t* test and analysis of variance (ANOVA) with PRISM (GraphPad Prism Software Inc., San Diego, CA, USA) [36]. Relative changes in cell count [%] were calculated as follows: relative changes in cell count [%] = ((mean B − mean A) ×

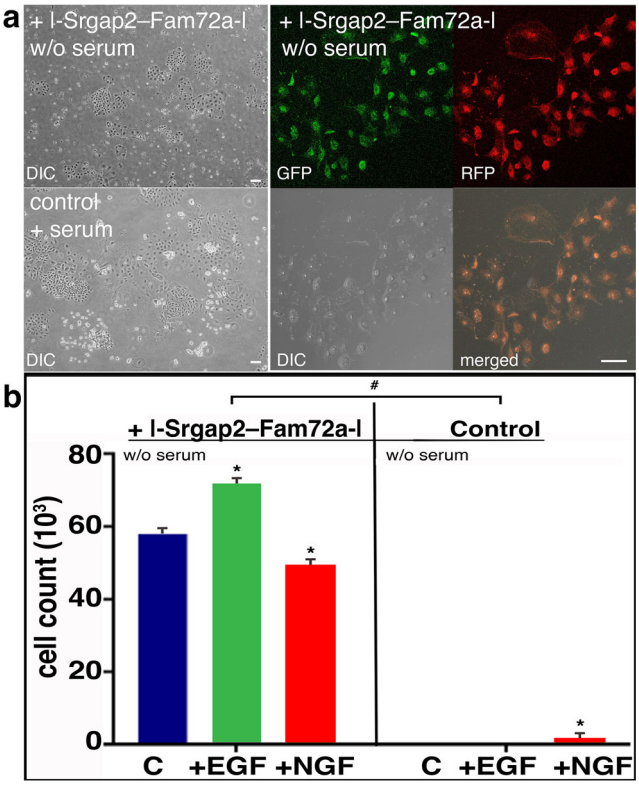


Fig. 3 Qualitative and quantitative comparison of |-Srgap2–Fam72a-|-transfected PC12 cells and non-transfected control PC12 cells. |-Srgap2–Fam72a-| DNA plasmid expressed either Srgap2 (RFP^+) or Fam72a (GFP^+) depending on IGR promotor activity. **a** PC12 cells were transfected with the |-Srgap2–Fam72a-| plasmid and incubated under serum-free conditions as indicated for 20 days. Pictures were taken every day, and representative pictures are shown (left hand: top (bright-field/DIC view): day 11, transfected PC12 cells, w/o serum, w/o Ngf/Egf treatment; left hand: lower picture (bright-field/DIC view): day 11, non-transfected control PC12 cells, with serum, w/o Ngf/Egf treatment). Right hand: representative fluorescence images of PC12 cells transfected with the |-Srgap2–Fam72a-| plasmid. Red fluorescence shows Srgap2 expression associated with neuronal survival. Green fluorescence indicates Fam72a transcription. Scale bar represents 50 μm. **b** FACS data showing (fluorescence-independent) cell counts from transfected and non-transfected PC12 cells after 14 days. |-Srgap2–Fam72a-|-transfected PC12 cells expressing Srgap2 and Fam72a survived significantly better than non-transfected PC12 cells under serum-free condition. |-Srgap2–Fam72a-|-transfected PC12 cells expressing Srgap2 and Fam72a w/o serum survived similar to non-transfected control PC12 cells cultivated with serum (**a**) while non-transfected cells could not survive under serum-free conditions except those treated with Ngf (**b**). $*p < 0.05$ compared with control cells (C); $^{\#}p < 0.05$, transfected cells compared with non-transfected cells; data are shown as mean ± SEM ($n = 5$)

100%)/(mean B), with mean A, mean of the number of fluorescence-positive ($^+$) cells of control |-Srgap2–Fam72a-|-transfected PC12 cells; and mean B, mean of the number of fluorescence$^+$ cells of treated (e.g., Ngf or Egf) |-Srgap2–Fam72a-|-transfected PC12 cell sample.

To be considered statistically significant, we required a p value of < 0.05 (95% confidence interval) from three to five independent experiments (n, number of independent experiments).

Results

Default |-Srgap2–Fam72a-| Master Gene Promotor IGR Setting—Effect of Basic Srgap2 and Fam72a Expression on PC12 Cells Under Control Conditions

|-Srgap2–Fam72a-|-transfected PC12 cells were analyzed by microscopy and FACS. Remarkably, |-Srgap2–Fam72a-|-transfected cells survived and proliferated without serum similar to non-transfected control PC12 cells with serum for up to 20 days (Fig. 3a, left hand, representative differential interference contrast (DIC) view after 11 days). Upon stimulation with Egf, the number of |-Srgap2–Fam72a-|-transfected PC12 cells increased while Ngf stimulation caused cell proliferation to stagnate. In non-transfected control PC12 cells cultivated without serum, the survival factor Ngf rescued a few cells (Fig. 3b).

Effect of Egf on |-Srgap2–Fam72a-|-Transfected PC12 Cells

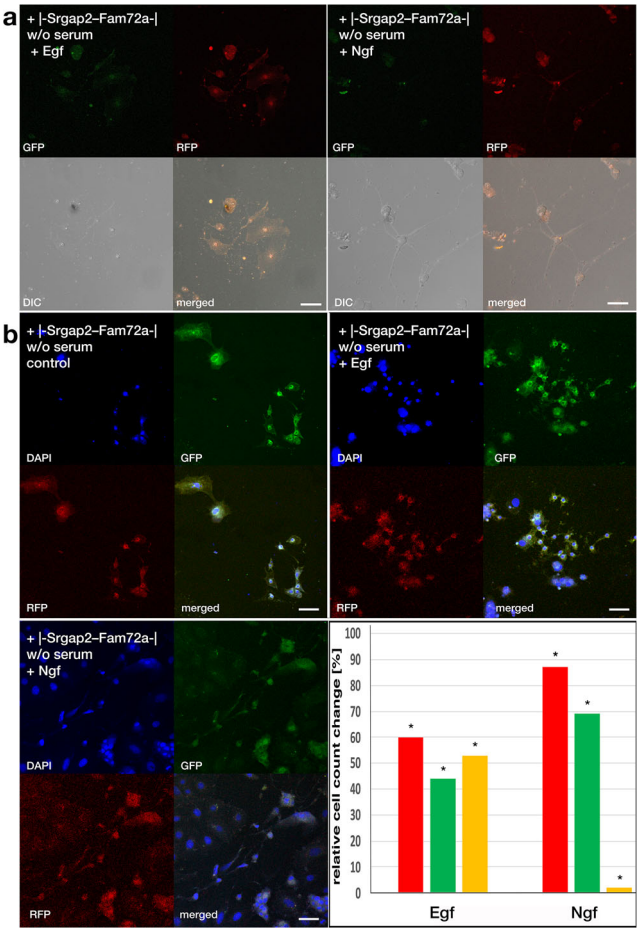
|-Srgap2–Fam72a-|-transfected PC12 cells were cultivated without serum, stimulated with mitogenic Egf for 14 days, and qualitatively analyzed by fluorescence microscopy (Fig. 4a). Green fluorescence indicated IGR element activation by Egf stimulation and was accompanied by Fam72a transcription and continuous cell proliferation (Fig. 4b). Red fluorescence, indicating Srgap2 transcription, was also visible (Fig. 4a, b).

Effect of Ngf on |-Srgap2–Fam72a-|-Transfected PC12 Cells

|-Srgap2–Fam72a-|-transfected PC12 cells were cultivated without serum, stimulated with Ngf for 14 days, and qualitatively analyzed by fluorescence microscopy (Fig. 4a). Red fluorescence indicated IGR promotor activation upon Ngf stimulation and was accompanied by Srgap2 transcription and neuritogenesis, which is typical of differentiated PC12 cells (Fig. 4a). Green fluorescence was still visible, indicating Fam72a expression (Fig. 4a, b).

IGR Acts as Dual Promotor to Control Srgap2 and Fam72a Expression

Quantitative comparison of Srgap2 and Fam72a expression in |-Srgap2–Fam72a-|-transfected PC12 cell by FACS analysis revealed that IGR acts as dual promotor to control Srgap2 and Fam72a transcription depending on the cellular input. A differentiation signal (i.e., Ngf) drove Srgap2 expression, neuronal differentiation, neurite outgrowth (Fig. 4a), and neuronal survival while an extracellular mitogenic signal (i.e., Egf)



◀ **Fig. 4** Qualitative and quantitative comparison of Srgap2 and Fam72a expression in |-Srgap2–Fam72a-|-transfected PC12 cells stimulated with Ngf or Egf. **a** Qualitative comparison of Srgap2 and Fam72a expression in |-Srgap2–Fam72a-|-transfected PC12 cells stimulated with Ngf or Egf. The |-Srgap2–Fam72a-| plasmid expressed either Srgap2 (RFP^+) or Fam72a (GFP^+) depending on IGR promotor activity. PC12 cells were transfected with the |-Srgap2–Fam72a-| plasmid and incubated w/o serum in the presence of either Ngf (100 ng/mL) or Egf (50 ng/mL) for 14 days. Red fluorescence indicates that Ngf promoted Srgap2 expression. Green fluorescence indicates Egf-mediated Fam72a transcription. Pictures were taken every day, and representative pictures from day 14 are shown. Scale bar represents 50 μm. **b** Quantitative comparison of Srgap2 and Fam72a expression in |-Srgap2–Fam72a-|-transfected PC12 cell by FACS analysis. The |-Srgap2–Fam72a-| plasmid expressed either Srgap2 (RFP^+) or Fam72a (GFP^+) depending on IGR promotor activity. PC12 cells were transfected with the |-Srgap2–Fam72a-| plasmid and incubated w/o serum in the presence of either Ngf (100 ng/mL) or Egf (50 ng/mL) for 10 days and were compared with untreated (w/o serum, w/o Ngf/Egf) |-Srgap2–Fam72a-|-transfected cells as control. Pictures were taken every day, and representative pictures for day 10 are shown. Blue, nuclear DAPI staining. Scale bar represents 50 μm. After 10 days, cells were collected for FACS analysis and relative changes in cell count [%] were calculated by comparing each subset of cells such as treated (Ngf or Egf) fluorescence$^+$ (RFP^+ or GFP^+ or both RFP^+ and GFP^+) population with its respective untreated control fluorescence$^+$ (RFP^+ or GFP^+ or both RFP^+ and GFP^+) population. The differentiation and survival factor Ngf induced an 87% increase in cells expressing Srgap2 (represented by the change of RFP^+ cells) and 67% increase in cells expressing Fam72a (represented by the change of GFP^+ cells). Data indicate that mitogenic Egf induced a 44% increase in cells expressing Fam72a (represented by the change of GFP^+ cells, i.e., the proportion of Fam72a expressing cells) and 60% increase in cells expressing Srgap2 (represented by the change of RFP^+ cells, i.e., the proportion of Srgap2 expressing cells). Yellow bars represent the relative change in double$^+$ cells expressing both Srgap2 and Fam72a (represented by the change of GFP^+ and RFP^+ cells, i.e., the proportion of Srgap2 and Fam72a expressing cells) upon Ngf or Egf stimulation. FACS results are presented as a percentage of the population normalized to the respective stimulated-transfected PC12 cells. $*p < 0.05$ comparing stimulated with un-stimulated cells within each subset of fluorescence$^+$ cells ($n = 4$)

drove Fam72a transcription (Fig. 4a, b) and continuous cell proliferation (Fig. 4b).

Discussion

Insight into control of the cell cycle and gene transcription is of utmost significance as it may open new therapeutic options for aging-related diseases such as dementia or cancer.

Two of the most important genes associated with cognition are Srgap2 and Fam72a, both of which appear on chr 1 (human) as a coupled gene set on opposite DNA strands. These genes occur four times in the human genome but only once in all other vertebrates [17, 26, 31]. This neural-specific gene set controls NSC proliferation and differentiation, and dysregulated expression affects the cell cycle causing serious diseases, such as cancer, in the brain and throughout the organism [17, 26, 27, 30, 37, 38].

Accumulating data points to our working hypothesis that Srgap2 plays a large role in neuronal differentiation and synaptic plasticity [19–23, 39, 40] while Fam72a is switched on (and Srgap2 off) during cell proliferation. Under physiological conditions, this mechanism allows for NSC self-renewal, and under pathological conditions, it can result in cancer [17, 24–27]. NSC self-renewal and differentiation depend on intrinsic and extrinsic factors and require strict control of gene expression, for which a dual promotor seems optimal [35, 41–44]. Gene sets, such as Srgap2 and Fam72a, must be under tight control of a dual-use master promotor (the IGR) with a reciprocal gene expression dependency. A |-Srgap2–Fam72a-| master gene with an IGR, containing a dual master promotor with two individual directional subpromotors, is the best method to control NSC renewal and differentiation. Post-translational modifications (including histone methylation, acetylation, and ubiquitination) appear to be involved in controlling two unidirectional core promotors (one each for Srgap2 and Fam72a) within the |-Srgap2–Fam72a-| master gene to direct the NSC toward self-renewal or irreversible neuronal differentiation [33, 34].

Because we expressed the rat |-Srgap2–Fam72a-| master gene from a plasmid, the lack of genomic chromatin/nucleosome structures or epigenetic directionality signals (e.g., genomic methylation) may have affected our results. However, the current study supported our hypothesis that Ngf-dependent Srgap2 expression was associated with

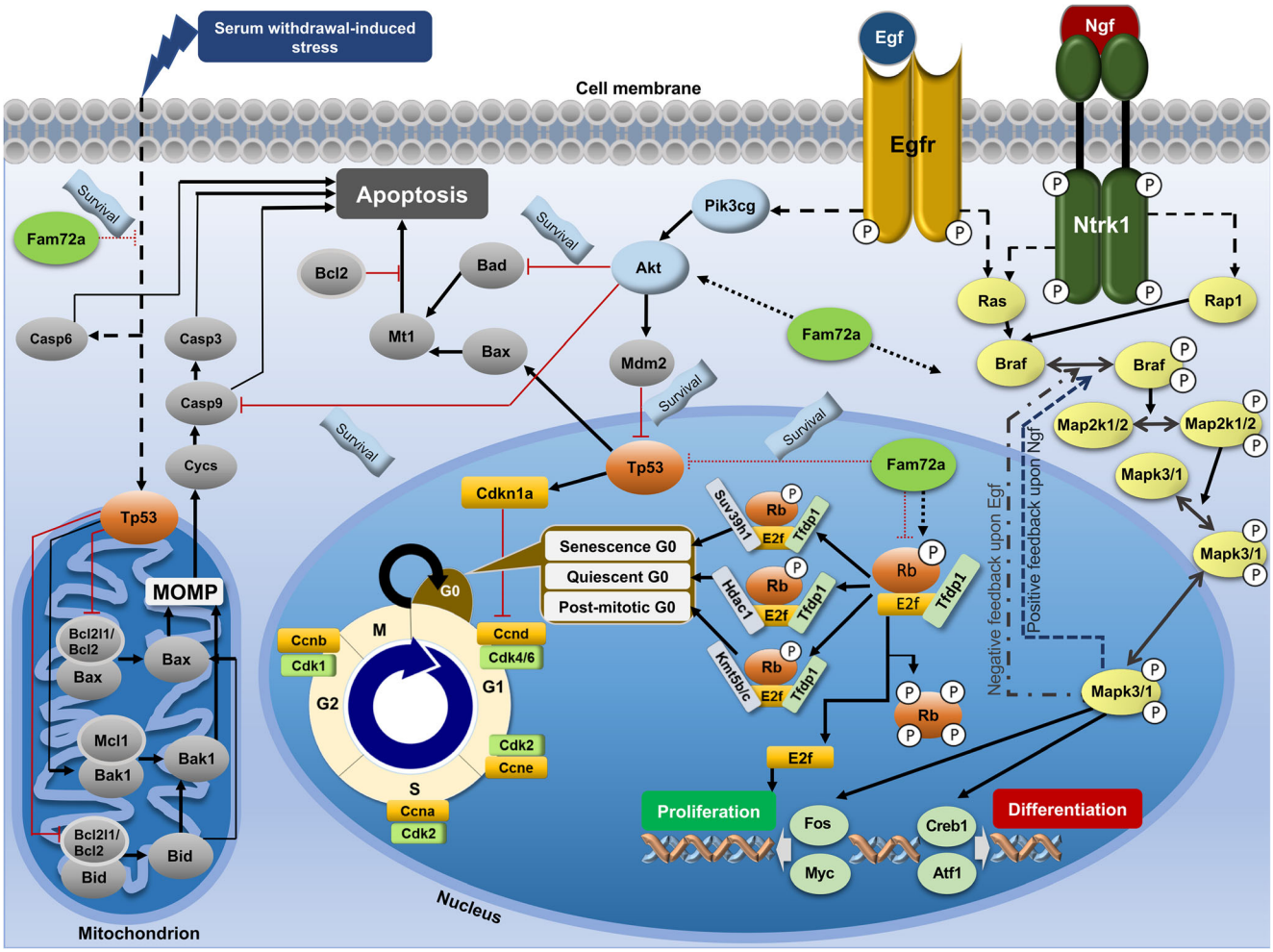


Fig. 5 Simplified overview of the effects of |-Srgap2–Fam72a-| master gene expression in PC12 cells. Under serum-free conditions, an early anti-apoptotic rescue program activates IGR-based expression of |-Srgap2–Fam72a-| to inhibit the pro-apoptotic Tp53/Casp3 pathways (denoted by dotted red lines), thus blocking the caspase pathways and driving the cell through the cell cycle to mediate further Fam72a expression. Fam72a in turn induces the pro-survival Akt and Mapk3/1 (mitogen-activated protein kinase 3/1, (Erk1/2, extracellular signal-regulated kinases)) cascades to enhance cell survival and proliferation [48–50]. A feedback loop further stabilizes and maintains Fam72a expression, thus modeling processes required for NSC self-renewal. Srgap2 expression maintains a neuronal phenotype by stabilizing neuronal survival at stage G0 while Fam72a affects Tp53 acetylation and Rb activity, which push cells into the G0 stage [14–16, 47, 51, 52]

neuronal survival, differentiation, and neurite outgrowth while Egf-dependent Fam72a transcription was associated with continuous cell proliferation (Figs. 4 and 5). Strikingly, under serum-free conditions, when PC12 cells usually undergo apoptosis [45–47], PC12 cells transfected with the |-Srgap2–Fam72a-| master gene survived and proliferated under serum-free conditions similar to non-transfected control PC12 cells cultivated with serum for a long time (Fig. 3a). This result further indicates that without serum, PC12 cells initiated an anti-apoptotic survival program that activated IGR which in turn kept Fam72a expression at sufficient levels to maintain cell cycle activity and a minimal Srgap2 activity to maintain a neuronal phenotype. Upon growth factor (Ngf or Egf)–mediated stimulation, Fam72a expression (in proliferating PC12 cells stimulated by Egf) or Srgap2 expression (in differentiating PC12 cells stimulated by Ngf) was enhanced; besides, Ngf-stimulated PC12 cells appeared to remain in a G0 stage co-expressing Srgap2 and Fam72a under IGR control without further proliferation (long-term effect, data not shown).

Conclusions

The present study demonstrated that an IGR serving as dual-use master promotor and reciprocal functional gene transcription dependency to optimize expression of the brain-specific, neural |-Srgap2–Fam72a-| master gene may serve as a model for future investigations on divergent gene transcription to further understand this novel gene expression paradigm.

Understanding the functional significance of the |-Srgap2–Fam72a-| master gene in NSC renewal/differentiation [3–7] and cancer cell proliferation [37, 38] may open new avenues to enhance cognitive function and treat diseases such as Alzheimer's disease or cancer.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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