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Peptide Regulation of Cell Differentiation

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Abstract
Short peptides are molecules with small molecular weight, capable of penetrating the cell membrane and nuclear membrane for epigenetic regulation of gene expression, including the genes responsible for cell differentiation. The direction of cell differentiation induction depends on the peptide structure and concentration. AEDG and AEDP peptides induce differentiation of pluripotent cells in the epidermis, mesenchyme and nervous tissue. Peptides KE, AED, KED, AEDG and AAAAEKAAAAEKAAAAEK activate neural differentiation. Peptides AEDL and KEDW induce lung and pancreatic cell differentiation. Differentiation of immune cells is stimulated by KE, DS, (Nα-(γ-E)-E), K(H-E-OH)-OH, AED, KED, EDA, and KEDG peptides. IRW, GRGDS and YCWSQYLCY peptides activate osteogenic differentiation of stem cells. KE, AEDL, and AEDG peptides also induce plant cells differentiation. Short peptides can take part in activation of the signaling pathways regulating expression of differentiation genes. They can interact with histones changing the availability of genes for transcription, regulate gene methylation and activate or inhibit their expression, as well as directly interact with the DNA. Research in the area of directed stem cell differentiation by peptide regulation is of special importance for developing innovative approaches to molecular medicine and cell therapy.

Keywords Short peptides · Epigenetics · Cell differentiation · Stem cells

Introduction

Cell differentiation is responsible for maintaining homeostasis during embryonic development and the following ontogenesis [1]. The pool of pluripotent cells found in virtually all human tissues can provide additional resources to inhibit the involution process [2, 3]. One of the manifestations of ageing is weakening of the differentiation ability of pluripotent cells. Short peptides are biologically active substances with small molecular weight capable of penetrating through the cytoplasm and nuclear membrane [4, 5]. They can epigenetically regulate expression of various gene groups including those responsible for cell proliferation, differentiation and apoptosis [6–8]. The aim of this review is to analyze the data obtained by the authors and available from literature concerned with the effects of short peptides on the differentiation of various cell types. Based on generalizing the review data, a possible mechanism for regulation of cell differentiation by peptides is suggested.

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Peptide Effects on Embryonic Pluripotent Cell Differentiation

The effect of AEDG (pineal gland functional regulator) and AEDP (cortex functional regulator) on pluripotent ectoderm tissue differentiation of clawed frog *Xenopus laevis* early gastrules has been studied. Portions of ectodermal blastocyst cavity tapetum of early *Xenopus laevis* gastrules were placed in the solution of AEDG and AEDP peptides in the final concentrations of 2, 10, 20, 50, 100, 200 ng/ml for 1 h. As controls, cell cultures were incubated in the Niu Vitti solution [9]. In the control cultures of *Xenopus laevis* pluripotent ectodermal tissue, only atypical epidermis developed. Under the impact of AEDG and AEDP peptides, the pluripotent cells were induced to differentiate into epidermis, mesenchyme

and neural tissues. The extent and direction of differentiation depended on peptide concentration.

In the presence of AEDP in concentration of 10 ng/ml, 80% of the pluripotent cells were induced to differentiate forming epidermis and mesenchyme. In concentrations of 2, 20, 100 ng/ml, the AEDP effect amounted to 30–48%. AEDG peptide in concentrations of 10, 50, 100 ng/ml equally stimulated differentiation of about 14% of pluripotent cells into neural tissue and epidermis. Thus, the peptide structure and concentration determine the direction of pluripotent cell differentiation [9].

Peptide Influence on Neuronal Differentiation of Stem Cells

Short peptides are capable of inducing neuronal differentiation of stem cells. The effect of KE, AED, KED and AEDG peptides, as well as that of their mixtures, has been studied in the final concentration of 100 ng/ml for neuronal differentiation of human periodontal ligament stem cells, hPDLSCs. The culture with no peptides added was used as the control. KE, AED, KED and AEDG peptides, as well as their mixture, enhanced synthesis of the marker of neuronal differentiation GAP43 in hPDLSCs culture as compared to the control. The largest effect was achieved for KED peptide and peptide mixture. According to the data obtained by immunocytochemistry, the neuronal marker of nestin precursors was expressed in over 50% of the differentiated hPDLSCs. Western blot analysis confirmed the results of immunohistochemistry, demonstrating increased GAP43 and nestin content in differentiated cells under the influence of peptide mixture as compared to that in non-differentiated cells without peptide treatment [10]. Moreover, AEDG and KED peptides prevent p16 and p21 gene expression and protein synthesis. These peptides could be used as supplementary substances in the culture medium to delay the expression of senescence markers in long term stem cell cultivation [11].

Earlier studies on neuronal cultures of murine hippocampus and corticosteroids in Alzheimer and Huntington disease models proved KED peptide to possess neuroprotective properties [12, 13]. The ability of KED peptide to induce neuronal differentiation could be one of the factors underlying its neuroprotective effect.

The KE peptide effect (0.05 ng/ml) on chicken retina cell differentiation was also compared to that of KE and AEDG peptide combination (0.01 ng/ml). To control the retina cell cultures, the saline was added. On the third day of cultivation, immunocytochemical analysis was performed with antibodies to retina cell differentiation markers, including Brn3, Pax6, Prox1, Vsx1 and TTP. KE peptide and KE + AEDG peptide mixture enhanced protein synthesis of retina cell differentiation protein markers 1.5–14.5 times as compared to the controls. KE peptide had larger effect on the expression of retina neuronal markers Brn3, Prox1 and Vsx1, while the KE and AEDG mixture induced cell marker expression of retina pigment epithelium TTR [8]. Earlier it had been shown that AEDG peptide inhibits development of pigment retinitis in rats of Campbell strain [14]. AEDG peptide effect on retina neuronal differentiation and on pigment epithelium is one of the mechanisms of its retinoprotective effect at experimental pigment retinitis [15].

Cell penetrating peptides (CPPs) also belong to short peptides. CPPs can penetrate the cell membrane and carry through other molecules. CPPs transport retinoic acid into the cell, which induces neuronal differentiation of stem cells [16–18]. As retinoic acid penetrates the cell membrane, it binds with the nuclear receptors RAR and RXR. RAR is heterodimerized using RXR and binds with proteins, which are part of the response to retinoic acid (RARE) and activate transcription factors for neurogenetic induction [19–21]. Synthetic CPP was studied, which is known as PepB (multiple repeats of AAAAEK aminoacid sequence) in complex with retinoic acid, in terms of its ability to penetrate into the cell and stimulate neuronal differentiation. ReNcell VM strain of neuronal precursors was chosen as a cell culture for study. The authors synthesized nine different PepB structures. Out of all the synthesized peptides, PepB3 demonstrated the strongest ability to penetrate the cell membrane (AAAAEKAAAAEKAAAAEK peptide), therefore further the authors analyzed the combination of PepB3 with retinoic acid (RA-PepB3) in different concentrations. The concentration under investigation was 0.1 umol/L, because in larger concentrations retinoic acid exhibits cell toxicity. After 4 weeks of ReNcell VM culture incubation with RA-PepB3, there was an increase in microtubular protein expression of β-III tubulin and neuronal protein MAP2. The authors explain the presence of PepB peptide with triple amino acid sequence by the peptides with alpha-spirals demonstrating the highest ability to penetrate the cell membrane. In order to form a stable alpha-spiral structure, minimum two to three coils are required, which amounts to 7–11 amino acid residues [22]. β-III tubulin and MAP2 expression in cells treated with RA-PepB3 complex, and those treated with retinoic acid alone, was at the same level. Thus, di-, tri- and tetrapeptides (KE, AED, KED, AEDG) stimulate neurogenic differentiation of stem cells, whereas the same effect of the longer peptides belonging to the CPP group (7–11 amino acid residues) remains to be proved.

Peptide Influence on Human Lung Cell Differentiation

AEDL peptide effect was studied on human embryonal lung cell differentiation (line FLECH) in the replicative ageing model. AEDL peptide was added to the experimental cell cultures in 20 ng/ml concentration. Physiological solution or KEDW peptide, which has pancreoprotective effect, was added to the cell culture as a control. To determine the peptide

influence on bronchial epithelium cell differentiation, quantitative polymerase chain reaction (qPCR) was used. This technique allowed determining *NKX2–1, SCGB1A1, SCGB3A2, FOXA1, FOXA2, MUC4, MUC5AC* and *SFTPA1* gene expression. AEDL peptide in 1.5 to 2.6 times increased *NKX2–1, SCGB1A1* and *SCGB3A2* gene expression of early human embryo lung cell differentiation, both in "young" and "mature" cells. This tetrapeptide stimulated *FOXA1* and *FOXA2* gene expression in "young", "mature" and "old" cells to increase 2–15 times. AEDL peptide activated *MUC5AC* and *SFTPA1* gene expression, whose inhibition correlates with lung pathology development. In "old" cell cultures of human embryo lungs, AEDL peptide activated the expression of genes participating in terminal differentiation and supporting functional cell activity. At the same time, the influence of this peptide on early differentiation genes was identified only in "young" and "mature" cultures. By means of spectrophotometry, viscosimetry and circular dichroism in vitro, interaction was shown between AEDL peptide with the DNA groove at N7 guanine. At the same time, KEDW peptide had no effect on the expression of genes, whose products participate in lung cell differentiation [23]. At replicative ageing of human lung cell culture and under AEDL peptide influence, the methylation profile of *PDX1, PAX6, NGN3, NKX2–1* and *SCGB1A1* gene promoter sites is changed, which correlates with the level of gene expression [24]. The influence of AEDL peptide on lung cell differentiation could underlie the bronchoprotective effect of the peptide in the models of inflammatory diseases, fibrosis and toxic lung damage in animals [23].

Peptide Influence on Pancreatic Cell Differentiation

KEDW peptide is a bioregulator of pancreatic functions. It stimulated human pancreatic cell differentiation in line MIA PaCa-2. KEDW peptide at 20 ng/ml concentration increased expression of *PDX1, NGN3, PAX6, FOXA2, NKX2.2, NKX6.1* and *PAX4* genes, as well as synthesis of the corresponding proteins in pancreatic cells. The results obtained using physical methods (UV-visible absorption spectroscopy and circular dichroism), as well as molecular modeling, suggest that KEDW peptide selectively binds with nitrogen DNA bases at the major groove [25, 26].

Peptide Influence on Immune Cell Differentiation

Short peptides can stimulate immune cell differentiation. Effects of (Nα-(γ-E)-E), K(H-E-OH)-OH, AED, and KEDG peptides at the concentration of 2, 20 and 200 ng/ml were studied on immune cell differentiation of human fetal bone marrow, thymus and liver, as well as peripheral blood of adults. H-K(H-E-OH)-OH) and KEDG peptides in concentrations of 2, 20 ng/ml stimulated differentiation of $CD34^+$ cell progenitors in fetal bone marrow and liver of embryons into myeloid $CD14^+$ cells, precursors of T-lymphocytes, mature T-helpers and cytotoxic T-cells. The most intensive stimulation of cell differentiation into mature T-lymphocytes and NK-cells was induced by KEDG peptides at 20, 200 ng/ml. Besides, (Nα-(γ-E)-E), K(H-E-OH)-OH, AED and KEDG peptides changed the differentiated human T cell prototype. Under the influence of these peptides mature blood T-lymphocytes changed their phenotype from $CD4^-CD8^+$ and $CD4^+CD8^-$ to $CD4^+CD8^+$ [27]. Notably, under the peptide influence, co-expression of the second receptor occurs on the membrane of the cell sub-population, which is indicative of their differentiation. Thus, the ability of the peptides was established to change cell differentiation.

Similar data were obtained by studying (Nα-(γ-E)-E), K(H-E-OH)-OH and AED peptide influence in the final concentration of 200 ng/ml on human embryo thymocytes (14 to 20 weeks of gestation) and naive thymocytes at the age of under 18 months. Under the influence of these peptides, at their replicative ageing in thymocyte culture, the proportion of $CD3^+CD4^+$ cells decreased by 65%, while that of $CD3^+CD8^+$ cells increased by 90–95%. As is known, at ageing thymocyte differentiation is shifted towards an increase in the number of $CD3^+CD4^+$ T-helpers and decrease in the number of $CD3^+CD8^+$ cytotoxic T-lymphocytes. It can be concluded that peptides activated cytotoxic T lymphocyte differentiation, which testifies to their geroprotective effect [6].

In another experiment, EDA and KED peptides stimulated $CD4^+CD8^+$ human embryo cortical thymocyte differentiation into $CD4^+CD25^+$ regulatory T-cells. Besides, under KED peptide influence the number of $CD4^+CD8^-$ T-helpers increased by 10%, whereas the number of $CD4^+CD8^+$ thymocytes decreased by 10% as compared to the controls [7].

Similar data were obtained for dipeptides. The influence of KE and DS peptides in final concentrations of 0.05 ng/ml on thymus cell cultures of 3-month-old rats (Wistar line) and those of human embryos was studied. KE peptide induced CD4 and CD5 molecule expression in thymus cells, stimulating their differentiation into T-helpers. DS peptide activated cytotoxic T lymphocyte differentiation, which was testified by increased expression of CD5 and CD8 markers in thymus cells [28].

Peptide Influence on Osteogenic Cell Differentiation

Data are also available on short peptide effect on osteogenic differentiation. Initially W9 (YCWSQYLCY) peptide was considered to be a factor inhibiting osteoclast differentiation. W9 peptide induced osteogenesis through osteoblasts of MC3T3-E1 line via activation of p38 MAPK and Smad1/5/8 pathways. At the same time, W9 effect was stronger than that of the bone morphogenic proteins (BMPs). W9 peptide also induced differentiation of osteoblasts in cells of MC3T3-E1 strain and in human mesenchymal stem cells (MSC) [29].

W9 peptide in concentrations of 100, 200 umol/l stimulated mineralization of stem cells obtained from human adipose tissues. The mineralization degree under the peptide impact was higher than that in the cell group treated with BMP2 or saline. Mineralization was enhanced with increasing peptide concentration. In the cell cultures studied by immunofluorescence, osteocalcin was found, which is an osteoblast marker. The largest numbers of osteocalcin-positive cells were present in the culture treated with W9 peptide, with fewer cells in a culture treated with BMP2, and no cells present in the controls. In the stem cells of adipose human tissue, BMP2 protein and W9 peptide activated expression of *TGFb1* gene, which is a ligand for transferring TGF-pathway signals, as well as the expression of *COL1A1* and *Runx2* genes, which are related to osteogenesis. W9 peptide had a larger effect on *COL1A1 and Runx2* activation as compared to BMP2 protein. BMP2 protein and W9 peptide decreased *BMPR1b* and *BMPR2* gene expression and increased expression of *TGFR1 and TGFR2* genes in stem cells of human adipose tissues. *BMPR1b* and *BMPR2* are BMP-signaling pathway mediators BMPs bind with receptor 2, type BMPR2, which induces BMPR1 activation. Further, BMPR1 is subject to phosphorylation. This is followed by activation of SMAD transcription regulator. BMP signaling pathway is responsible for osteogenesis and bone cell differentiation. Simultaneous decrease in *BMPR1b* and *BMPR2* expression and increase in *TGFR1 and TGFR2* expression under the influence of BMP2 protein and W9 peptide proves that in stem cells of human adipose tissue these factors trigger differentiation along *TGFR1 and TGFR2* pathways.

W9 peptide and BMP2 protein activated phosphorylation of p38 and Erk1/2 molecules in stem cells. Addition of W9 peptide caused JNK and Akt protein phosphorylation in cell cultures. Activation of signal transmission via Akt and JNK appears to be necessary for W9-induced osteogenic differentiation of stem cells in human adipose tissue, which makes this signaling pathway different from BMP2-induced osteogenesis [30].

Another research group studied the influence of GRGDS peptide immobilized on titanium dioxide nanotube on the differentiation of osteoblast-like cell line MG-63. This study is of special importance since the surfaces of titanium implants, which have gained good reputation in dentistry, react with oxygen to form titanium oxide [31]. GRGDS peptide stimulated osteoblast adhesion, proliferation and differentiation. Titanium dioxide nanotube immobilized with GRGDS peptide can be used in dentistry to improve osteo-integration of tooth implants [32].

The effect of IRW peptide was also studied on differentiation of osteoblasts, line MC3T3-E1. IRW peptide is a bioactive molecule obtained from hydrolyzate of the ovotransferrin protein. IRW peptide is biologically active in a wide range of areas, including antihypertensive [33], anti-inflammatory [34] and antioxidative [35] effects. Considering the data that prove inflammation and oxidative stress to play an important role in bone formation [36, 37], it was suggested that IRW peptide could be used to regulate osteoblast differentiation. By adding IRW peptide to MC3T3-E1 cell line in concentrations 25, 50 umol/l, the phosphorylation of serine was enhanced 4 times in Akt-kinase, and that of RUNX2 protein, which was Akt-kinase target, was enhanced twice. IRW peptide twice enhanced the synthesis of ALP (Alkaline phosphatase), Col1A2 (Collagen, type I, alpha 2) and OPG (Osteoprotegerin), while, at the same time, decreasing two times the synthesis rate of RANKL protein (Tumor necrosis factor ligand superfamily member 11). ALP is a side product of osteoblast activity. ALP level grows at active osteogenesis of CollA2, which is one of the chains of fibrillary collagen, type 1. OPG plays an important part in bone tissue metabolism as a RANKL receptor in the RANK/RANKL/OPG axis by inhibiting osteoclastogenesis and bone resorption [38]. RANKL, in its turn, is the key factor in the differentiation and activation of osteoblasts, which are responsible for bone tissue resorption. Enhancing ALP, Col1A2 and OPG synthesis and inhibiting RANKL synthesis under the influence of IRW is indicative of a stimulating osteogenic effect of the latter peptide (Fig. 1).

At the same time, IRW peptide activates collagen synthesis, which is also an important stage of osteogenesis. Thus, IRW peptide enhances bone tissue osteoblast activity and stimulates osteogenic cell differentiation. Stimulation of osteogenic cell differentiation under the influence of IRW peptide is mostly due to the activation of PI3K-Akt-RUNX2 pathway and its subordinate effectors, accompanied by collagen synthesis enhancement [39].

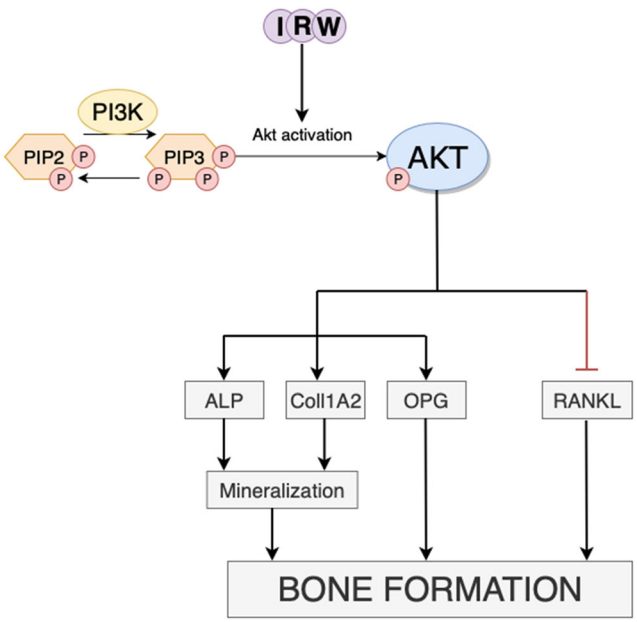

Fig. 1 Effect of IRW peptide on osteogenic differentiation through influencing PI3K-Akt signaling pathway (modified according to [39])

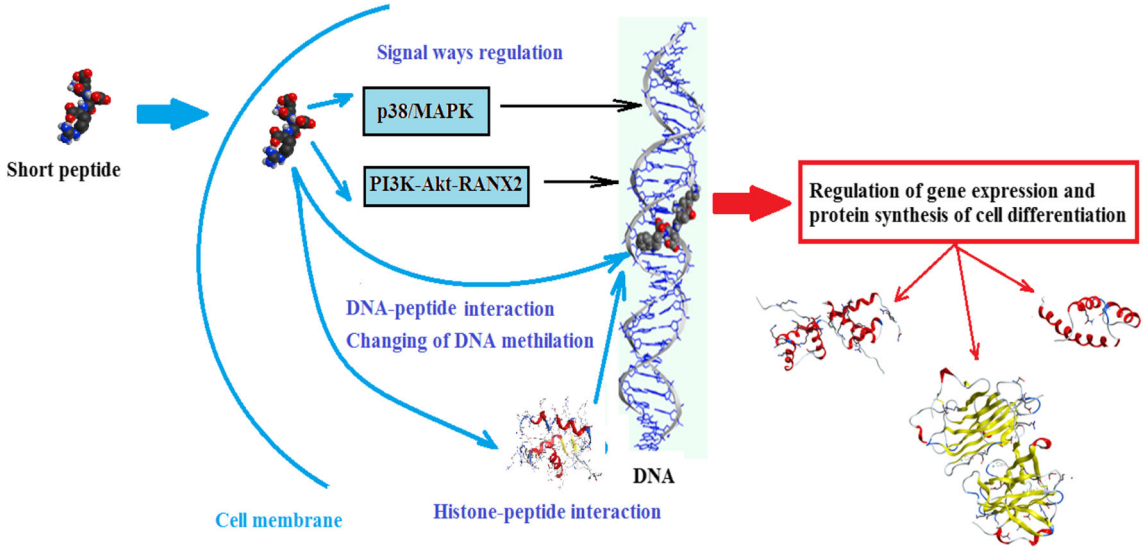

Fig. 2 Possible ways of cell differentiation regulation by peptides

It can be suggested that short peptides induce osteogenic cell differentiation by activating p38/MAPK and PI3K-Akt signaling pathways. Activation of these pathways can take place via binding peptides with signaling cascade elements or via their ability to regulate expression of genes that encode p38/MAPK and PI3K-Akt protein signaling pathways.

Peptide Influence on Plant Cells Differentiation

Besides the stimulating effect on human and animal cell differentiation, short peptides regulate expression of genes concerned with plant cells differentiation. The effect was studied of KE, AEDG and AEDL peptides on the expression of *CLE, KNOX1* and *GRF* gene family responsible for the differentiation of tobacco plant cells (*Nikotiana tabacum).* The genes of *CLE* family encode endogenic regulatory plant peptides, the genes of *KNOX1* family encode transcription factors, while *GRF* genes are growth factor regulators encoding DNA-binding proteins. When growing tobacco calluses on standard medium with KE, AEDG and AEDL peptides added in the concentrations of 1–100 nmol/l, increased callus growth was observed as compared to the controls. From our own perspective, the effect of KE, AEDL and AEDG peptides on the genes of *KNOX1* family seems of most interest, because the genes of this family are critical regulators of stem cell homeostasis in plants, encoding the transcription factors responsible for inhibition of cell differentiation in the seedling apex. *KNOX1 Nikotiana tabacum* family includes *KNAT1, KNAT2, KNAT6, LET6* and *LET12 genes*. All these genes take part in stem cell differentiation. Adding KE and AEDG peptides induced a double increase in *KNAT3* gene expression, while adding AEDL peptide increased it 4 times. *KNAT6* gene expression rose 4 to 6 times under the influence of the peptides concerned. In other plants, arbidopsis in particular, STM gene is homologous to *LET6 and LET12* genes. This gene is known to be responsible for leaf differentiation. Considering the data available on the effect of AEDG and AEDL peptides on leaf formation and growth, it can be suggested that AEDL peptide

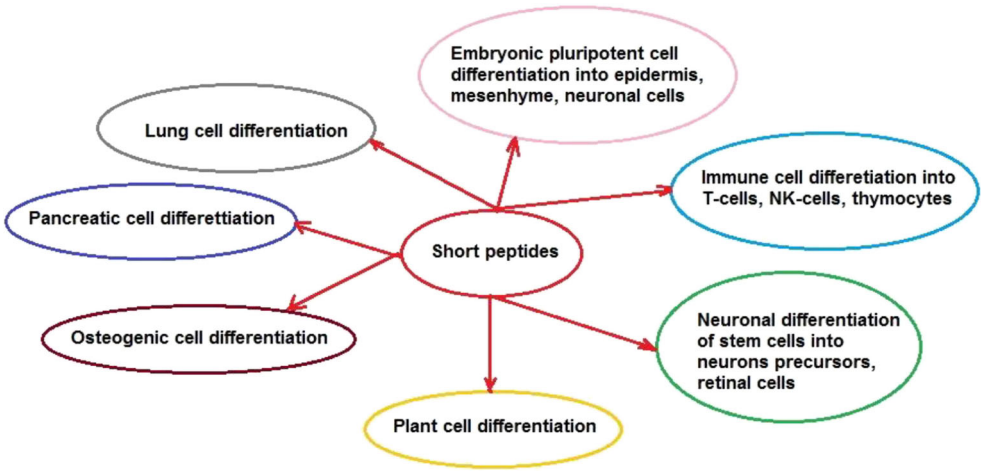

Fig. 3 Directions of cell differentiation under the short peptide impact

makes the largest contribution to leaf differentiation. Apparently, due to having similar structure, AEDG peptide can produce the same stimulating effect. Underlying the mechanism of peptide effect on *Nikotina tabacum* cell differentiation could be their binding with the DNA and histones. For AEDL and AEDG peptides, the target for binding in histones could be the positively charged KAAKAKK motif, while that for KE peptide, the negatively charged EVAA motif. It is suggested that specific binding of short peptides to histones can modulate the action of various enzymes on chromatin histones and have a marked influence on the multiple known enzymatic histone "tails". As they modify chromatin, the interactions of short peptides with histones can serve as a mechanism for epigenetic regulation of genetic activity, including cell differentiation [40]. It can be suggested that regulation of plant, animal and human cell differentiation by peptides is of similar nature.

Conclusion

The data available from literature and obtained by us allow suggesting the following mechanism of cell differentiation regulation by peptides (Fig. 2). Short peptides penetrate the cell cytoplasmic membrane, and further four options are possible. Peptides can take part in the activation of signaling pathways which regulate differentiation of gene expression, by interacting with histone proteins, changing gene accessibility for transcription, regulating gene methylation status and activating/inhibiting their expression or directly interacting with the DNA [23, 25–27, 39, 40]. An example of signaling pathway activation by peptides is induction of osteogenic cell differentiation due to the impact of IRW, GRGDS and YCWSQYLCY peptides on p38/MAPK and PI3K-Akt-RUNX2 cascade. AEDL and KEDW peptides regulate gene methylation status by interacting with certain DNA sequences, which induces lung and pancreatic cell differentiation. According to the data obtained by molecular modeling and physiochemical research, KE, AED, KED and AEDG peptides interact with DNA and histone proteins, facilitating immunogenic and neurogenic stem cell differentiation.

The effect of short peptides on cell differentiation depends on the peptide structure and concentration. AEDG and AEDP peptides induce pluripotent cell differentiation into epidermis, mesenchyme and nervous tissue. KE, AED, KED, AEDG and AAAAEKAAAAEKAAAAEK activate neuronal differentiation. AEDL and KEWD peptides induce lung and pancreatic cell differentiation, respectively. Immune cell differentiation is stimulated by peptides KE, DS, (Nα-(γ-E)-E), K(H-E-OH)-OH, AED, KED, EDA and KEDG. IRW, GRGDS and YCWSQYLCY peptides activate osteogenic stem cell differentiation. KE, AEDL and AEDG also induce plant cell differentiation. Notably, induction of various directions of stem cell differentiation by short peptides (Fig. 3) is of high practical significance for treating age-related diseases and preventing accelerated ageing [41].

Thus, depending on their structure and concentration, short peptides epigenetically regulate gene expression and protein synthesis by activating cell differentiation in various directions. Short peptides carry great promise are for application in different areas of molecular medicine and could be prospectively used in cell therapy of various diseases.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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