Chemerin Promotes The Proliferation And Migration of Breast Cancer Cells Through JNK And ERK1/2 Signaling pathways

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Abstract

Breast cancer is the most common invasive malignancy. In 2020, the number of new cases of breast cancer worldwide has replaced lung cancer as the No.1 cancer in the global. Breast cancer is the leading cause of cancer death among women worldwide. Mammary tumorigenesis is severely linked to obesity, one potential connection is chemerin. Chemerin is a chemoattractant protein secreted by adipocytes, which contributes to the progression of breast cancer. Cell proliferation, migration, and invasion are cellular processes associated with various stages of metastasis. These processes are associated with mitogen-activated protein kinase (MAPK) signaling pathway. In this study, human breast cancer cell lines MCF-7 and MDA-MB-231 were utilized to determine the effect of chemerin on cell proliferation, migration, and key proteins of MAPK signaling pathway. We found that chemerin promoted cell proliferation and migration in a concentration-dependent manner. Interestingly, these effects of chemerin were through promoting the proteins phosphorylation of ATF2 and ERK1/2 but not p38, in MAPK signaling pathway. Specific inhibitors of JNK and ERK1/2 pathway showed that the effect exerted by chemerin in cell proliferation and migration in breast cancer cells was dependent on these proteins. Our findings suggest that chemerin promotes the development of mammary cancer cells through JNK and ERK signaling pathways.

Introduction

Breast cancer is the most common malignancy in women worldwide, accounting for a quarter of cancer cases in women[1], and causing nearly 700,000 deaths globally each year[2]. According to the data released by the World Health Organization's International Agency for Research on Cancer (IARC) in 2020, the number of new cases of breast cancer worldwide has reached 2.26 million, which has replaced lung cancer as the No.1 cancer in the global[3]. Breast cancer is the leading cause of cancer death among women worldwide in 2020, accounting for 680,000 deaths[3]. There are many important risk factors for breast cancer, including obesity, estrogen, and so on[4]. Obesity, which is a worldwide epidemic[5], has been found to be associated with different kinds of cancers[6]. Post-menopausal obesity has especially given the increased risk of breast cancer[7]. It has been reported that post-menopausal obesity with an excess estrogen condition can cause a 63% increasing risk in breast cancer [6] through many biologically active factors including estrogen, leptin and chemerin, ect[8–11]. Chemerin, discovered adipokine recently, also known as tazarotene-induced gene 2 (TIG2) or retinoic acid receptor responder 2 (RARRES2), is a chemoattractant protein which was demonstrated to regulate adipogenesis, metabolism, immunity and tumorigenesis[12–15]. It has been closely associated with metabolic syndrome, obesity, inflammatory diseases and cancer[16–25]. At present, the relationship between chemerin and cancer is not clearly understood completely. The conflicting reports, tumorigenic[25–27] and antitumor[12, 28, 29] effects, about the connection between chemerin and cancer, have caused this relation to be controversial. For breast cancer, some suspect that chemerin may be involved in a higher risk, albeit indirectly[30]. Another research[10] has showed that chemerin expression is positively relevant to patients' body weight, body mass index (BMI), tumor size, metastasis including lymph node metastasis and distant metastasis, and

tumor grading, respectively, and is inversely relevant respectively to estrogen receptor (ER) and progesterone receptor (PR) expression in breast cancer tissues. Chemerin expression is a predictor and prognostic factor for breast cancer. While a cross sectional study[5] showed that chemerin concentration in serum could not be used in breast cancer stage by studying 117 patients with breast cancer. Researchers found that chemerin concentrations in serum were no differences between patients with metastatic and non-metastatic cancer. These studies have an important limitation which was short of a control group without breast cancer. Moreover, some adipose-derived angiogenic factors have shown experimentally that they may promote breast cancer growth, though this has yet to be tested in the context of chemerin[31, 32]. It has also been conjectured that chemerin may play a role in promoting metastasis through its angiogenic functions.

In this study, we evaluated the roles of chemerin in cell proliferation and migration in a cultured cell model of breast cancer, and its related mechanism. We utilized the human breast cancer cell lines MCF-7 and MDA-MB-231 and found that chemerin promoted cell proliferation and migration by up-regulating proteins phosphorylation of ATF2 and ERK1/2 of MAPK pathway but not p38. Using a combination of inhibitors for these proteins we found a decrease in cell proliferation and migration in breast cancer cells. Here, we presented that chemerin contributed to cell proliferation and migration in breast cancer cell lines by the molecular mechanism of the signaling events which the chemerin system may be utilized or manipulated for clinical benefit.

1. Materials And Methods

1.1 Materials

Recombinant human chemerin were obtained from Peprotech (USA). Chemerin neutralization antibody was purchased from Beijing Bosen Biology Co. Ltd (China). Inhibitors of JNK (SP600125), ERK1/2 (FR180204) and p38 (SB203580) were obtained from Shanghai Lanmu Chemical Co. Ltd (China). All kinds of antibodies including rabbit anti-ATF-2 and anti-ERK1 & ERK2 and anti-p38 antibodies, the phospho-specific antibodies against ATF-2, ERK1 & ERK2, and p38 were obtained from Abcam (USA).

1.2 Cell line and culture

Human breast cancer cell lines MCF-7 & MDA-MB-231 were purchased from Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China), and were cultured in RPMI-1640 (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Hyclon, USA) and 1000U/mL Penicillin & 100µg/mL streptomycin (Beijing Solaibao Technology Co. Ltd, China). Cells were incubated at 37°C with 5% CO₂ under a humidified atmosphere. The following procedures were applied to all assays (i.e., cell proliferation assay, cell wound healing assay and western blot analyses). Breast cancer cell lines MCF-7 and MDA-MB-231 were cultured in serum-free medium for 18h. Then, along with the control (without any treatment), the following concentrations of chemerin (1, 10 and 100µg/L) were added to the respective wells. The cells were incubated with chemerin for 24 h to test cell proliferation. And concentrations of

chemerin(100µg/L) or chemerin neutralizing antibody (100µg/L) were used to test cell proliferation & migration and protein expressions.

1.3 Cell proliferation assay

The MTT assay was used to evaluate the effect of chemerin on cell proliferation. MCF-7 & MDA-MB-231 cells were cultured in T-75 culture flasks, up to 85% confluency. Cells were harvested and then seeded in 96-well plates overnight at a density of 5×10^3 cells per well contain RPMI-1640 medium 200µL. Afterwards, cells were grown in serum-free medium for 18h, chemerin (Peprotech, USA) with varying concentrations of 1, 10 and 100µg/L or chemerin neutralizing antibody (100µg/L) (Beijing Bosen Biology Co. LTD, China) was then added to the medium. The control group was treated with an equal volume of RPMI-1640 with 10% FBS. After 24 h, the cells were incubated with MTT (5mg/ml) 20µL for 4 h, then lysed in dimethylsulfoxide (DMSO) 150µL for 30min. Finally, the optical density (OD) at 490nm was measured with a microplate reader (Bio-Tek Instruments, Winooski, VT, USA). Cell proliferation assays were performed at least 3 times for each cell line (in replicates of 6 wells for each concentration of chemerin in each experiment). Data are presented as means $\pm$ SD for a representative experiment for each cell line.

1.4 Cell wound healing assays

MCF-7 & MDA-MB-231 cells were grown until confluence on 6-well culture plates supplemented with RPMI-1640 as described earlier. Then cells were scratch-wounded using a sterile 10µL pipette tip, suspended cells were removed by washing with PBS three times, and the cells were re-fed with RPMI-1640 in the presence or absence of the chemerin (1, 10, 100µg/L), chemerin neutralizing antibody (100µg/L), and pathway inhibitors (25mM/mL). The progress of cell migration into the wound was monitored at 0, 12 or 24, and 24 or 48 h using an Olympus inverted microscope with a 10× objective. The bottom of the plate was marked for reference, and the same point of the monolayers were photographed immediately after performing the wound (time=0 h, T0), 12 or 24, and 24 or 48 h after treatments. Then each of the scratch width was measured. Three plates of each group were analyzed. The migration rate represents cell migration ability which was calculated as (original scratch width – present scratch width)/original scratch width $\times$ 100%[33].

1.5 Western blot analysis

Cells were cultured in 6-well plates in serum-free medium, then treated with chemerin (100µg/L) or chemerin neutralizing antibody (100µg/L) and incubated for 24h following the above-mentioned protocol. For extraction of total soluble proteins from the cultured cells, highly efficient RIPA tissue/cell lysates (Beijing Solaibao Technology Co. Ltd, China) were used. Cell lysates were sonicated and subsequently centrifuged at 12,000rpm for 20min at 4°C, and clear supernatants were collected. For the Western blot analysis, 10% SDS-PAGE was used and proteins were transferred to PVDF membranes (Immobilon-P, Millipore, Billerica, MA). Primary antibodies including phospho-specific antibodies against the following proteins were used for probing: ATF-2, ERK1 & ERK2 and p38 (all antibodies were from Abcam, USA). Mouse anti-actin antibody was from Beijing Zhongshan Jinqiao Biological Co. Ltd, China. Suitable

secondary IgGs conjugated with horseradish peroxidase (HRP) were used as secondary antibody. Proteins were detected by Chemiluminescence Detection kit (Beijing Kangwei Biological Co. Ltd, China) and analyzed the gray values of the obtained bands by Gel imaging system (Bio-Rad, USA).

1.6 Statistical analysis

Results are presented as means $\pm$ standard error of the mean (SEM) for a representative experiment for each cell line. Data were statistically analyzed using GraphPad Prism version 5 software (GraphPad Software, Inc., CA, USA). Analysis of more than two groups was performed using one-way ANOVA and the pairwise comparisons using *t* test. Difference at $p < 0.05$ was considered significant.

2 Results

2.1 Chemerin promotes cell proliferation in a concentration-dependent manner

Breast cancer cells MCF-7 and MDA-MB-231 were treated with different concentrations of chemerin (1, 10, 100 μ g/L). After 24h, the cell proliferation was detected by MTT method. The results showed that OD value of each concentration of chemerin treatment group was higher than that of the control group ($p < 0.05$, Fig. 1). The higher the concentration of chemerin, the bigger OD value is. The promotion effect of 100 μ g/L chemerin treatment group was the most obvious.

2.2 Chemerin promotes cell migration in a concentration-dependent manner

MCF-7 and MDA-MB-231 breast cancer cells were treated with different concentrations of chemerin (1, 10, 100 μ g/L), and cell migration ability was detected by scratch assay. The results showed that after 48 h of scratching, the cell migration rate of each concentration of chemerin treatment group was higher than that of the control group ($p < 0.05$ or $p < 0.01$, Fig. 2). The higher the concentration of chemerin, the stronger the promotion effect of cell migration is. The promotion effect of 100 μ g/L chemerin treatment group was the most obvious.

2.3 Chemerin neutralizing antibody inhibits cell proliferation

Breast cancer cells MCF-7 and MDA-MB-231 were treated with chemerin (100 μ g/L) and chemerin neutralizing antibody (100 μ g/L), respectively. The cell proliferation was detected by MTT assay. The results showed that compared with the control group, the OD value of MCF-7 and MDA-MB-231 breast cancer cells was significantly increased after chemerin was treated for 24h, while the OD value of the chemerin neutralizing antibody group was lower than that of the control group. This indicated that chemerin neutralizing antibody could significantly reduce the proliferation ability of breast cancer cells ($p < 0.05$ or $p < 0.01$, Fig. 3).

2.4 Chemerin neutralizing antibody inhibits cell migration

Breast cancer cells MCF-7 and MDA-MB-231 were treated with chemerin (100µg/L) and chemerin neutralizing antibody (100µg/L), respectively. Cell migration was detected by scratch assay. The results showed that 48h after scratching, the cell migration rate of the control group was significantly lower than that of the chemerin treatment group, while the cell migration rate of the chemerin neutralizing antibody group was significantly lower than that of the control group ($p < 0.05$, Fig. 4).

2.5 Chemerin enhances protein ATF2 and ERK1/2 phosphorylated expressions but no change for p38

MCF-7 and MDA-MB-231 breast cancer cell lines were cultured and respectively treated with chemerin (100µg/L) and chemerin neutralizing antibody (100µg/L), the total protein was extracted after 24 h. ATF2, ERK1/2 and p38 proteins of JNK, ERK1/2 and p38 signaling pathway was detected by Western blot. The results showed that in MCF-7 cells, the protein expression levels of total ATF2, ERK1/2 and p38 had no significant differences compared with the control group. The phosphorylation of ATF2 and ERK1/2 proteins was significantly increased after chemerin treatment, but significantly decreased in the chemerin neutralizing antibody group ($p < 0.05$). The expression of phosphorylated p38 protein was not significantly changed either being treated by chemerin or chemerin neutralizing antibody (see Fig. 5A & B). Similar results were occurred in MDA-MB-231 cells (see Fig. 5A& C).

2.6 Effects of signaling pathway inhibitors on the ability of chemerin to promote the migration of breast cancer cells

Two breast cancer cell lines, MCF-7 and MDA-MB-231, were cultured and seeded into 6-well plates. After the cells were filled, the scratch experiment was performed. Before treated with signaling pathway inhibitors, each well was added with chemerin (100µg/L). Then, equal amounts of DMSO, JNK signaling pathway inhibitor SP600125, ERK1/2 signaling pathway inhibitor FR180204 and p38 signaling pathway inhibitor SB203580 were respectively added into different well. Samples were taken at 0, 24 and 48 h under an inverted microscope. Three sites were taken from each group for recording and cell migration rate was calculated. The results showed that the cell migration rate in groups of JNK and ERK1/2 signaling pathway inhibitor group were significantly smaller than that in the DMSO group ($p < 0.001$). The cell migration rate was smaller in the p38 signaling pathway inhibitor group, but there was no statistically different (Fig. 6).

3 Discussion

Breast cancer is the most common malignant tumor in women in the world at present. Many bioactive substances, for example leptin, which produced by adipose tissue, are closely related to the incidence of breast cancer. Chemerin is a protein produced by adipose tissue and is encoded by the RARRES2 gene, which translates into a protein containing 163 amino acids with a molecular weight of 18.618KDa[34, 35]. This protein is synthesized by adipose tissue triggered by TNF- α [36] and consists of a 20-amino acid

hydrophobic N-terminal signal peptide, an intervening 137-amino acid cysteine folded domain and a 6-amino acid pre-C-terminal fragment, with low activity[37]. Chemerin is the endogenous ligand of chemokine-like receptor 1 (CMKLR1) or ChemR23 (also known as DEZ), which is one of G-protein-coupled receptor, and plays a biological role by binding to these receptors. A number of studies have shown that chemerin plays a role in a various of pathophysiological processes such as obesity[18, 38], inflammatory[39, 40], metabolic syndrome[41], psoriasis[42], dilated cardiomyopathy[43], non-alcoholic steatohepatitis[44], and polycystic ovary syndrome[45]. At the same time, chemerin is also one of the key molecules involved in the process of oncogenesis and immune surveillance. The expression level of chemerin will also change during the occurrence and development of different cancers. Specifically, chemerin can promote or inhibit tumor occurrence and development[10, 16, 20, 46–48]. Studies have confirmed [49–52] that the major binding receptor of chemerin, CMKLR1, is expressed on the surface of immune cells and various malignant tumor cells. Second atypical receptor of chemerin, chemokine CC-motif receptor-like 2(CCRL2), is mainly expressed in endothelial cells, macrophages, dendritic cells, and so on. This Helps focus chemerin on activated endothelial cells and sites of inflammation. Though chemerin has not been deeply studied in breast cancer, there are several preliminary pieces of evidence showing that chemerin may have a tumor-promoting effect. Chemerin is known to be associated with obesity and obesity-related parameters, such as blood pressure and BMI, and post-menopausal obesity is associated with an increased risk of breast cancer, so some researchers suspect that chemerin may be correlated to a higher risk of breast cancer, albeit with indirect evidence [30]. It has also been speculated that chemerin may play a role in instigating metastasis through its angiogenic functions. However, in breast adenocarcinoma, which is one of the most common types of breast cancer, the expression of chemerin is significantly down-regulated [12], suggesting that chemerin may have an antitumor effect in breast cancer. This anti-tumor role was also verified by Kim[53]. Our study showed that OD value and cell migration rate increased with the increase of chemerin concentration in a concentration-dependent manner after the treatment of MCF-7 & MDA-MB-231 human breast cancer cell lines with different concentrations of chemerin. Moreover, after treatment with chemerin neutralizing antibody, the OD value and cell migration rate of the cancer cells were reduced. These results suggest that chemerin can promote the proliferation and migration of breast cancer cells. Our findings support the tumor-promoting effect of chemerin.

Chemerin is a chemotactic protein that acts as a ligand for both CMKLR1, one of the G-protein-coupled receptors, and GPR1 and CCRL2. Only the biological actions of chemerin/CMKLR1 axis have been clearly described at present[39, 54–56]. CMKLR1 is known to be expressed on the surface of a variety of malignant tumor cells [49–52]. The stimulation of chemerin can induce cell invasion of human mesenchymal stromal cells[19], gastric cancer cells[20], and esophageal squamous cancer cell[26]. In patients with advanced stage of non-small cell lung cancer (NSCLC) receiving platinum-based chemotherapy, the SNP rs1878022, located within an intron in the CMKLR1 gene on chromosome12q23.3, was positively statistical associated with decreased overall survival rate [57]. This suggests that CMKLR1 could have a role in NSCLC, and in vitro studies are in agreement. Study has shown that chemerin activates key mitogen-activated protein kinase (MAPK) and Akt signaling pathways

and stimulates gelatinolytic activity of matrix metalloproteinase (MMP) to induce cell migration and angiogenesis[58]. The MAPK signaling pathway is one of the original signaling systems which exists in all eukaryotes, and controls fundamental cellular processes such as proliferation, differentiation, survival and apoptosis. Based on the structure and function, mammalian MAPKs can be divided into three groups: extracellular signal-regulated kinases (ERKs), p38 MAPKs, and c-Jun NH2-terminal kinases (JNKs). Activation of these MAPKs occurs through a cascade of upstream kinases. Our study showed that cell migration was significantly inhibited after MCF-7 & MDA-MB-231 human breast cancer cell lines treated by JNK and ERK pathway inhibitors, while cell migration was not significantly affected by treatment with p38 pathway inhibitor. These results indicate that chemerin plays roles through JNK and ERK signaling pathways, not the P38 signaling pathway.

Although our study has showed that chemerin promotes breast cancer growth through JNK and ERK signaling pathways, it is still unclear whether chemerin can be used as therapeutic target or biomarker for breast cancer and it needs in-depth study by a large number of researchers.

Declarations

Acknowledgement

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Authors' Contributions

Study concept and design: Luo N, Zeng F, Zhang J; Acquisition of data: Zhang J, Zeng F, Zhou X, Liu Q, Yang S, Luo N; Analysis and interpretation of the data: Luo N, Zeng F, Zhang J, Liu Q, Zhou X. Write the paper: Luo N, Zeng F, Zhang J. All authors reviewed the manuscript before submission.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors, therefore no ethic approval or consent is required. No administrative permission and/or licenses is acquired by this study to access the original data used in this research.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no conflict of interest in this study.

References

1. M. Ghoncheh, Z. Pournamdar, H. Salehiniya. Incidence and Mortality and Epidemiology of Breast Cancer in the World[J]. *Asian Pac J Cancer Prev*. 2016, 17(S3):43–46.
2. J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012[J]. *Eur J Cancer*. 2013, 49(6):1374–1403.
3. H. Sung, J. Ferlay, R.L. Siegel, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries[J]. *CA Cancer J Clin*. 2021, 71(3):209–249.
4. R. Qureshi, M. Picon-Ruiz, I. Aurrekoetxea-Rodriguez, et al. The Major Pre- and Postmenopausal Estrogens Play Opposing Roles in Obesity-Driven Mammary Inflammation and Breast Cancer Development[J]. *Cell Metab*. 2020, 31(6):1154–1172.
5. B. Cabia, S. Andrade, M.C. Carreira, et al. A role for novel adipose tissue-secreted factors in obesity-related carcinogenesis[J]. *Obes Rev*. 2016, 17(4):361–376.
6. A. Booth, A. Magnuson, J. Fouts, et al. Adipose tissue, obesity and adipokines: role in cancer promotion[J]. *Horm Mol Biol Clin Investig*. 2015, 21(1):57–74.
7. E. Heer, A. Harper, N. Escandor, et al. Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study[J]. *Lancet Glob Health*. 2020, 8(8): e1027-e1037.
8. B. Lauby-Secretan, L. Dossus, C. Marant-Micallef, et al. [Obesity and Cancer][J]. *Bull Cancer*. 2019, 106(7-8):635–646.
9. E. Guastamacchia, F. Resta, V. Triggiani, et al. Evidence for a putative relationship between type 2 diabetes and neoplasia with particular reference to breast cancer: role of hormones, growth factors and specific receptors[J]. *Curr Drug Targets Immune Endocr Metabol Disord*. 2004, 4(1):59–66.
10. G. El-Sagheer, M. Gayyed, A. Ahmad, et al. Expression of chemerin correlates with a poor prognosis in female breast cancer patients[J]. *Breast Cancer (Dove Med Press)*. 2018,10:169–176.
11. C. Garofalo, E. Surmacz. Leptin and cancer[J]. *J Cell Physiol*. 2006, 207(1):12–22.
12. R.K. Pachynski, B.A. Zabel, H.E. Kohrt, et al. The chemoattractant chemerin suppresses melanoma by recruiting natural killer cell antitumor defenses[J]. *J Exp Med*. 2012, 209(8):1427–1435.
13. Y. Song, W. Yin, Y. Dan, et al. Chemerin partly mediates tumor-inhibitory effect of all-trans retinoic acid via CMKLR1-dependent natural killer cell recruitment[J]. *Immunology*. 2019, 157(3):248–256.
14. C. Buechler, S. Feder, E.M. Haberl, et al. Chemerin Isoforms and Activity in Obesity[J]. *Int J Mol Sci*. 2019, 20(5).

15. F. Eichelmann, C. Weikert, R. di Giuseppe, et al. Methodological utility of chemerin as a novel biomarker of immunity and metabolism[J]. *Endocr Connect*. 2017, 6(5):340–347.
16. Z. Lu, J. Liang, Q. He, et al. The serum biomarker chemerin promotes tumorigenesis and metastasis in oral squamous cell carcinoma[J]. *Clin Sci (Lond)*. 2019, 133(5):681–695.
17. N. Wang, Q.J. Wang, Y.Y. Feng, et al. Overexpression of chemerin was associated with tumor angiogenesis and poor clinical outcome in squamous cell carcinoma of the oral tongue[J]. *Clin Oral Investig*. 2014, 18(3):997–1004.
18. G. Rowicka, H. Dyląg, M. Chelchowska, et al. Serum Calprotectin and Chemerin Concentrations as Markers of Low-Grade Inflammation in Prepubertal Children with Obesity[J]. *Int J Environ Res Public Health*. 2020, 17(20):7575.
19. P. Vinci, A. Bastone, S. Schiarea, et al. Mesenchymal stromal cell-secreted chemerin is a novel immunomodulatory molecule driving the migration of ChemR23-expressing cells[J]. *Cytotherapy*. 2017, 19(2):200–210.
20. J.D. Kumar, I. Aolymat, L. Tiszlavicz, et al. Chemerin acts via CMKLR1 and GPR1 to stimulate migration and invasion of gastric cancer cells: putative role of decreased TIMP-1 and TIMP-2[J]. *Oncotarget*. 2019, 10(2):98–112.
21. K. Landgraf, D. Friebe, T. Ullrich, et al. Chemerin as a mediator between obesity and vascular inflammation in children[J]. *J Clin Endocrinol Metab*. 2012, 97(4):E556-564.
22. P. Kulig, T. Kantyka, B.A. Zabel, et al. Regulation of chemerin chemoattractant and antibacterial activity by human cysteine cathepsins[J]. *J Immunol*. 2011, 187(3):1403–1410.
23. S. Schultz, A. Saalbach, J.T. Heiker, et al. Proteolytic activation of prochemerin by kallikrein 7 breaks an ionic linkage and results in C-terminal rearrangement[J]. *Biochem J*. 2013, 452(2):271–280.
24. X. Qi, J. Fan, J. Zhu, et al. Circulating chemerin level and risk of cancer: a systematic review and meta-analysis[J]. *Biomark Med*. 2020, 14(10):919–928.
25. V. Farsam, A. Basu, M. Gatzka, et al. Senescent fibroblast-derived Chemerin promotes squamous cell carcinoma migration[J]. *Oncotarget*. 2016, 7(50):83554–83569.
26. J.D. Kumar, S. Kandola, L. Tiszlavicz, et al. The role of chemerin and ChemR23 in stimulating the invasion of squamous oesophageal cancer cells[J]. *Br J Cancer*. 2016, 114(10):1152–1159.
27. C. Tümmler, I. Snapkov, M. Wickström, et al. Inhibition of chemerin/CMKLR1 axis in neuroblastoma cells reduces clonogenicity and cell viability in vitro and impairs tumor growth in vivo[J]. *Oncotarget*. 2017, 8(56):95135–95151.
28. K. Rennie, W.J. Shin, E. Krug, et al. Chemerin Reactivates PTEN and Suppresses PD-L1 in Tumor Cells via Modulation of a Novel CMKLR1-mediated Signaling Cascade[J]. *Clin Cancer Res*. 2020, 26(18):5019–5035.
29. R.K. Pachynski, P. Wang, N. Salazar, Y. Zheng, et al. Chemerin Suppresses Breast Cancer Growth by Recruiting Immune Effector Cells Into the Tumor Microenvironment[J]. *Front Immunol*. 2019, 10:983.

30. K. Bozaoglu, K. Bolton, J. McMillan, et al. Chemerin is a novel adipokine associated with obesity and metabolic syndrome[J]. *Endocrinology*. 2007, 148(10):4687–4694.
31. A.M. Lorincz, S. Sukumar. Molecular links between obesity and breast cancer[J]. *Endocr Relat Cancer*. 2006, 13(2):279–292.
32. S. Perrier, F. Caldefie-Chézet, M.P. Vasson. IL-1 family in breast cancer: potential interplay with leptin and other adipocytokines[J]. *FEBS Lett*. 2009, 583(2):259–265.
33. T. Huang, W.H. Gong, X.C. Li, et al. Efficient killing effect of osteosarcoma cells by cinobufacini and cisplatin in combination[J]. *Asian Pac J Cancer Prev*. 2012, 13(6):2847–2851.
34. W. Meder, M. Wendland, A. Busmann, et al. Characterization of human circulating TIG2 as a ligand for the orphan receptor ChemR23[J]. *FEBS Lett*. 2003, 555(3):495–499.
35. V. Wittamer, J.D. Franssen, M. Vulcano, et al. Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids[J]. *J Exp Med*. 2003, 198(7):977–985.
36. S.D. Parlee, M.C. Ernst, S. Muruganandan, et al. Serum chemerin levels vary with time of day and are modified by obesity and tumor necrosis factor- α [J]. *Endocrinology*. 2010, 151(6):2590–2602.
37. A. Mattern, T. Zellmann, A.G. Beck-Sickinger. Processing, signaling, and physiological function of chemerin[J]. *IUBMB Life*. 2014, 66(1):19–26.
38. E.M. Haberl, R. Pohl, L. Rein-Fischboeck, et al. Ex vivo analysis of serum chemerin activity in murine models of obesity[J]. *Cytokine*. 2018, 104:42–45.
39. J. Shang, L. Wang, Y. Zhang, et al. Chemerin/ChemR23 axis promotes inflammation of glomerular endothelial cells in diabetic nephropathy[J]. *J Cell Mol Med*. 2019, 23(5): 3417–3428.
40. L.J. Cajas Santana, F. Rondón Herrera, A.P. Rojas, et al. Serum chemerin in a cohort of Colombian patients with primary osteoarthritis[J]. *Reumatol Clin (Engl Ed)*. 2020, S1699-258X(20)30136-4.
41. H.J. Ba, L.L. Xu, Y.Z. Qin, et al. Serum Chemerin Levels Correlate With Determinants of Metabolic Syndrome in Obese Children and Adolescents[J]. *Clin Med Insights Pediatr*. 2019, 13: 1179556519853780.
42. M. Coban, L. Tasli, S. Turgut, et al. Association of Adipokines, Insulin Resistance, Hypertension and Dyslipidemia in Patients with Psoriasis Vulgaris[J]. *Ann Dermatol*. 2016, 28(1):74–79.
43. D. Chen, J. Wang, J. Fu. Serum Chemerin Predicts the Prognosis of Patients With Dilated Cardiomyopathy[J]. *Heart Surg Forum*. 2020, 23(3):E276-E280.
44. L. Zhang, X.F. Gao, Y.H. Wang. [The expression of chemerin and the influence of sitagliptin on its expression in non-alcoholic fatty liver disease rats complicated with prediabetes][J]. *Zhonghua Yi Xue Za Zhi*. 2018, 98(30):2407–2413.
45. A.A. Foda, E.A. Foda, M.A. El-Negeri, et al. Serum chemerin levels in Polycystic Ovary Syndrome after metformin therapy[J]. *Diabetes Metab Syndr*. 2019, 13(2):1309–1315.
46. G.P. Sotiropoulos, M. Dalamaga, G. Antonakos, et al. Chemerin as a biomarker at the intersection of inflammation, chemotaxis, coagulation, fibrinolysis and metabolism in resectable non-small cell lung

- cancer[J]. Lung Cancer. 2018, 125:291–299.
47. C.H. Xu, Y. Yang, Y.C. Wang, et al. Prognostic significance of serum chemerin levels in patients with non-small cell lung cancer[J]. Oncotarget. 2017, 8(14):22483–22489.
  48. M.M. Alkady, P.L. Abdel-Messeih, N.M. Nosseir. Assessment of Serum Levels of the Adipocytokine Chemerin in Colorectal Cancer Patients[J]. J Med Biochem. 2018, 37(3):313–319.
  49. J.L. Cash, R. Hart, A. Russ, et al. Synthetic chemerin-derived peptides suppress inflammation through ChemR23[J]. J Exp Med. 2008, 205(4):767–775.
  50. S. Perumalsamy, N.A. Aqilah Mohd Zin, R.T. Widodo, et al. Chemokine Like Receptor-1 (CMKLR-1) Receptor: A Potential Therapeutic Target in Management of Chemerin Induced Type 2 Diabetes Mellitus and Cancer[J]. Curr Pharm Des. 2017, 23(25):3689–3698.
  51. W. M, Z. W, G. J. Wortmannin inhibits the activity of NLRP3 in Kupffer cells through the Chemerin/CMKLR1 pathway and relieves nonalcoholic fatty liver in mice[J]. Chongqing Medicine. 2018, 47(17):2279–2284.
  52. N. Nakamura, K. Naruse, Y. Kobayashi, et al. Chemerin promotes angiogenesis in vivo[J]. Physiol Rep. 2018, 6(24):e13962.
  53. H. Kim, J.H. Lee, S.K. Lee, et al. Chemerin Treatment Inhibits the Growth and Bone Invasion of Breast Cancer Cells[J]. Int J Mol Sci. 2020, 21(8):2871.
  54. B. Bondue, V. Wittamer, M. Parmentier. Chemerin and its receptors in leukocyte trafficking, inflammation and metabolism[J]. Cytokine Growth Factor Rev. 2011, 22(5-6):331–338.
  55. X. Wang, J. Guo, Q. Wu, C. N, et al. Chemerin/chemR23 association with endothelial-mesenchymal transition in diabetic nephropathy[J]. Int J Clin Exp Pathol. 2017, 10(7):7408–7416.
  56. L. Rouger, G.R. Denis, S. Luangsay, et al. ChemR23 knockout mice display mild obesity but no deficit in adipocyte differentiation[J]. J Endocrinol. 2013, 219(3):279–289.
  57. X. Wu, Y. Ye, R. Rosell, et al. Genome-wide association study of survival in non-small cell lung cancer patients receiving platinum-based chemotherapy[J]. J Natl Cancer Inst. 2011, 103(10):817–825.
  58. J. Kaur, R. Adya, B.K. Tan, et al. Identification of chemerin receptor (ChemR23) in human endothelial cells: chemerin-induced endothelial angiogenesis[J]. Biochem Biophys Res Commun. 2010, 391(4):1762–1768.

Figures

Figure 1

Cell proliferation assay after 24h chemerin treatment. The OD values of breast cancer cells MCF-7 treated with different concentrations of chemerin (1, 10, 100µg/L) were all higher than those of the normal control group. Breast cancer cells MDA-MB-231 had similar results. *p<0.05

Figure 2

Effect of chemerin on migration of breast cancer cells. Breast cancer cells MCF-7 and MDA-MB-231 were treated with chemerin (1, 10, 100µg/L). The migration ability of the two cells at 0, 24 and 48 h was detected by scratch assay. The migration rate of breast cancer cells MCF-7 after chemerin treatment in the three groups was higher than that in the control group. Breast cancer cells MDA-MB-231 had similar results. *p<0.05, **p<0.01

Figure 3

Cell proliferation assay after 24 h chemerin or chemerin neutralizing antibody treatment. The OD values of both MCF-7 and MDA-MB-231 breast cancer cells after being treated with chemerin (100µg/L) were higher than that of the control group (p<0.01) while the OD values of the chemerin neutralizing antibody group were lower than that of the control group (p<0.05). *p<0.05, **p<0.01

Figure 4

Effect of chemerin neutralizing antibody on migration of breast cancer cells. The migration of breast cancer cells MCF-7 and MDA-MB-231 after treatment with chemerin (100µg/L) and chemerin neutralizing antibody (100µg/L) was detected at 0, 24 and 48 h. The cell migration rate of chemerin treatment group in MCF-7 was significantly higher than that of the control group ($p<0.001$), and the cell migration rate of chemerin treatment group in MDA-MB-231 was also higher than that of the control group ($p<0.05$). The cell migration rate of the chemerin neutralizing antibody group was lower than that of the control group both in breast cancer cells MCF-7 and MDA-MB-231 ($p<0.05$). * $p<0.05$, *** $p<0.001$

Figure 5

Protein expression of ATF2, ERK1/2 and p38 in breast cancer cells treated with chemerin (100 μ g/L) and chemerin neutralizing antibody (100 μ g/L). The protein expression levels of total ATF2, ERK1/2 and p38 in both MCF-7 and MDA-MB-231 cells were no significantly different among the three groups. The phosphorylation of ATF2 and ERK1/2 proteins was significantly increased after chemerin treatment, but significantly decreased in the chemerin neutralizing antibody group both in MCF-7 and MDA-MB-231 cells.

(A, B, C) . There was not statistically significant in the expression of phosphorylated p38 protein among the three groups both in MCF-7 and MDA-MB-231 cells(A, B, C). * $p < 0.05$

Figure 6

Effects of JNK, ERK1/2 and p38 signaling pathway inhibitors on the ability of chemerin to promote the migration of breast cancer cells. MCF-7 and MDA-MB-231 cells were treated with DMSO, JNK signaling pathway inhibitor SP600125, ERK1/2 signaling pathway inhibitor FR180204 and P38 signaling pathway inhibitor SB203580, respectively, and the cell migration rates were observed at 0, 24 and 48 h. The

migration rate in groups of JNK and ERK1/2 signaling pathway inhibitor group in MCF-7 and MDA-MB-231 cells were significantly smaller than that in the DMSO group ($p<0.001$), but there was no statistical significance between the p38 signaling pathway inhibitor group and the DMSO group. *** $p<0.001$

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