Original Article

Blocking the Notch signal transduction pathway promotes tumor growth in breast cancer by promoting the expression of suppressible inflammatory factors

Shuguang Ren[1,2], Yueyang Hu[1], Yingchao Ju[1], Yang Sang[1], Jianhua Wu[1,2], Yiming Ge[3], Beichen Liu[2,4], Xiaohan Zhao[2,5], Xiangmei Zhang[2,6], Yunjiang Liu[2,7]^

[1]Department of Animal Center, The Fourth Hospital of Hebei Medical University, Shijiazhuang, China; [2]Hebei Provincial Key Laboratory of Tumor Microenvironment and Drug Resistance, Hebei Medical University, Shijiazhuang, China; [3]School of Basic Medicine, Hebei Medical University, Shijiazhuang, China; [4]Department of Hematology, The Fourth Hospital of Hebei Medical University, Shijiazhuang, China; [5]Department of Radiotherapy, The Fourth Hospital of Hebei Medical University, Shijiazhuang, China; [6]Research Center, The Fourth Hospital of Hebei Medical University, Shijiazhuang, China; [7]Department of Breast Center, The Fourth Hospital of Hebei Medical University, Shijiazhuang, China

Contributions: (I) Conception and design: S Ren, Y Liu; (II) Administrative support: Y Hu, Y Ju; (III) Provision of study materials or patients: Y Sang; (IV) Collection and assembly of data: S Ren, Y Ge, B Liu, X Zhao, X Zhang, Y Hu; (V) Data analysis and interpretation: S Ren, J Wu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Yunjiang Liu. Department of Breast Center, The Fourth Hospital of Hebei Medical University, Shijiazhuang 050011, China. Email: lyj818326@outlook.com.

Background: Breast cancer is the most common malignant tumor among all female tumors. It seriously affects the health and lives of patients, and poses a significant economic burden. The study of the molecular mechanisms of breast cancer occurrence, proliferation and growth and development is of great clinical significance.

Methods: Notch1 knockout mice were obtained by gene targeting. The expression of inflammatory factor arginase-1 in each group of tumors was observed by immunofluorescence staining. Semi-quantitative detection of Notch1, Arginase-1, and proteins belonging to the PI3K-AKT pathway by western blot. The expression level of interleukin-3 (IL-3), and IL-4 in serum was quantified by enzyme linked immunosorbent assay (ELISA).

Results: In this study, Notch1 knockout in mice promoted the cell proliferation of breast cancer. Further study on molecular mechanisms demonstrated that the increased cell proliferation resulted from the activation of the PI3K-AKT signal transduction pathway. In addition, the expression of the M2-type inflammatory factor arginase-1 significantly increased, which was dependent on the activation of the PI3K-AKT pathway, indicating that Notch1 knockout in mice promoted the polarization of tumor-associated macrophages (TAMs). Consistent with this, IL-3 and IL-4 expression also significantly increased in the serum of Notch1 knockout mice.

Conclusions: According to our results, Notch1 knockout in mice significantly promoted the cell proliferation of breast cancer, not only by activating the PI3K-AKT pathway, but also by promoting the polarization of TAMs towards the M2-type phenotype.

Keywords: Notch1; tumor-associated macrophages (TAMs); M2-type; inhibitory factor; breast cancer

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^ ORCID: 0000-0001-7202-2004.

Introduction

The most common malignant tumor in women is breast cancer, which is a serious threat to the lives and health of women worldwide, and poses a serious economic burden. According to data obtained from the International Agency for Research on Cancer (IARC), it is estimated that by 2030, breast cancer cases and deaths in 69 countries around the world are likely to reach 2.64 million and 1.7 million, respectively (1). In China, according to the *2019 National Cancer Report*, the annual incidence rate of breast cancer is about 304,000, ranking the highest in terms of the incidence rate of female malignant tumors. At present, targeted therapy drugs have been developed for human epidermal growth factor receptor 2 (HER-2) positive, estrogen receptor (ER) positive, and progesterone receptor (PR) positive breast cancer patients, which greatly improves the survival time and quality of life of patients (2,3). Subsequently, targeted therapy drugs were developed for BRCA1/2 (4). However, some patients do not have a clear pathogenic mutation gene, known as triple negative breast cancer, which has the highest degree of malignancy. At present, conventional treatment methods include surgery, chemotherapy, and radiotherapy. For these patients, the study of its pathogenesis and the development of targeted drugs have become important research topics.

It has been proven that monocytes/macrophages are recruited towards the tumor microenvironment through neovascularization to form tumor-associated macrophages (TAMs) in the early stage of tumorigenesis. In this physiological process, the Notch signal transduction pathway regulates not only the production of recruitment factors by tumor cells, but also the differentiation of TAMs. Chemokine (C-C motif) receptor 2 (CCR2) is a key molecule involved in monocyte recruitment. The classical Notch signal transduction pathway interacts with CCR2/Chemokine (C-C motif) 2 (CCL2) of TAMs in the tumor microenvironment and mediates the localization of monocytes in tumor tissues in a CCR2-dependent manner during the early stage of tumorigenesis (5). In basal-like breast cancer, the Notch signal transduction pathway directly regulated the expression of CCL2 and interleukin-1β (IL-1β), which were Produced by tumor cells and bone marrow stromal cells (6). The secreted cytokines/chemokines contribute to monocyte recruitment to tumor tissue through monocyte adhesion to blood vessels and extravasation to tumor tissues. According to the research, Notch signaling pathway promoted ATM conversion to M1-type ATM to activate inflammatory response and subsequently inhibit the tumor growth of osteosarcoma.

The phenotype of TAMs in the tumor microenvironment is highly plastic. Macrophages polarize to 2 opposite functional phenotypes in pro-inflammatory (M1-type ATM) and anti-inflammatory (M2-type ATM) microenvironments. M1-type ATM releases pro-inflammatory cytokines to inhibit tumor growth; M2-type ATM can inhibit the release of inflammatory cytokines to promote tumor growth, of which one of the main features of M2-type ATM is the up-regulation of Arginase-1 (Arg-1), indicating the polarization of ATM to M2-type ATM. It was found that human breast cancer cells secreted macrophage colony stimulating factor (M-CSF) to induce TAMs towards an anti-inflammatory phenotype (7). In an *in vivo* model of breast cancer, the monocyte precursors differentiated into different TAM subpopulations (8). In addition, a study on renal cell carcinoma showed that TAMs had mixed pro-inflammatory and anti-inflammatory phenotypes (9). These results indicate that the phenotype of TAMs depends on the tumor, and their activation is highly dependent on the microenvironment, in which the Notch signal transduction pathway participates in TAM activation and promotes their differentiation into a pro-inflammatory phenotype. The Notch signal transduction pathway directly or indirectly promotes the transformation towards a pro-inflammatory phenotype of TAMs and regulates the transcription of some inflammatory factors, such as IL-6, IL-12B, and NOS2 (10). However, blocking it may lose some major pro-inflammatory functions and reduce the production of proinflammatory cytokines (such as IL-6) and nitric oxide (NO) (11).

Based on its role in TAM recruitment and differentiation, as well as its subsequent function in tumor suppression, the Notch signal transduction pathway is expected to become a drug target for tumor treatment. Various types of γ-secretase inhibitors are commonly used to inhibit the Notch signal transduction pathway in clinical trials. In addition, antibody-based specific blocking therapy for ligand binding regions or negative regulatory regions of Notch has also become a research hotspot in recent years (12). The purpose of this study is to discuss the role and mechanism of Notch1 in the occurrence and development of breast cancer. Our results demonstrated that blocking the Notch signal transduction pathway promoted tumor growth through activating the expression of IL-3 and IL-4. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-1041/rc).

Methods

Construction of Notch1 knockout mice

The mouse strain used in the study was B6.129X1-Notch1, which was purchased from (Strain #: 007181) The Jackson Laboratory. The mice were fed at 21–27 ℃ with freely drinking water and eating. The specific step of the construction of Notch1 knockout mice is to use gene targeting technology to insert two loxP gene sequences into both ends of the Notch1 gene, and then transfer them into embryonic stem cells in the uterus of pregnant mice. Notch1 gene knockout was detected by polymerase chain reaction (PCR). A protocol was prepared before the study without registration. The experiment was carried out under the project license issued by the Experimental Animal Ethics Committee of the Fourth Hospital of Hebei Medical University (No. IACUC-4th Hos Hebmu-20180212) and in line with the national guidelines on animal care and use.

Establishment of a tumor-bearing mouse model

Five pairs of 6-week-old mice with homologous Lyz2Cre Notch1 f/f and Lyz2Cre Notch1 +/+ were selected. The FCY20201MCF-7 of breast cancer in logarithmic phase was digested with trypsin and prepared into cell suspension. After disinfecting the skin of the mice with alcohol, FCY20201MCF-7 cell suspension was injected into the middle and lower part of the right axilla of the mice. The injection density was 2.5×10^7/mL and the injection volume was 0.2 mL of each mouse. Isoflurane gas anesthesia were used for reducing mice' pain. After the injection, the mice were put back into the cage and fed routinely. The living conditions of tumor-bearing mice were observed every day. After 2 weeks of treatment, the tumor growth was evaluated by small animal living imager. After 21 days, the tumor was removed and weighed. In addition, the effects of Notch1 gene knockout on tumor growth were compared.

Western blot

Total proteins were extracted from tumor tissues with RIPA buffer (Sigma, St. Louis, USA) and used for Western blot. The samples were sampled according to the amount of 30 μg protein per well, the proteins were separated by SDS-PAGE gel electrophoresis, and then transferred to PVDF membrane. Five percent skimmed milk powder (OXOID: LP0031B) was closed and incubated with an antibody (Cell Signaling, LA, USA).The second antibody was goat anti-mouse antibody(Jackson Labs, LA, USA) labeled with horseradish peroxidase and finally developed with ECL kit. Images represent 5 mice in 1 group.

Immunofluorescence staining

Cut the tumor tissue into small pieces and quickly fix it overnight with 10% neutral formalin solution. Gradient dehydration in 20% and 30% sucrose solution. Next, the dehydrated tissue was embedded with optimal cutting temperature compound (OCT), and the tissue section was carried out in the frozen slicer.Use adhesive slides to adhere to tissue slices and dry them in a constant temperature oven.Put into the citric acid antigen repair solution of PH 6.0, repair at 92 ℃ for 30 minutes, and then naturally cool to room temperature. H_2O_2 is used to block peroxidase in the tissue, and 3% BSA solution blocks natural antibodies. After tissue incubation, the first antibody was incubated overnight in a refrigerator at 4 ℃. The second antibody was incubated at room temperature for 1 hour the next day, and the nucleus was restained with Hoechst and then anti-fluorescence attenuation sealing tablet.

Enzyme linked immunosorbent assay (ELISA)

The ELISA kits were bought from Andy Gene Company (Andy Gene, USA) to measure the contents of brain derived neurotrophic factor (BDNF) (No. AD3269Ra), IL-6 (No. AD3249Ra), SOD (No. AD2871Ra), CAT (No. AD2899Ra), MMP-9 (No. AD3038Ra), IL-10 (No. AD3254Ra), and IFN-γ (No. AD3257Ra) in serum or hippocampi according to the manufacturer's instructions.

Statistical analysis

The column graphs were generated by GraphPad Prism software using the results from 3 biological replicates, and statistically significant differences were evaluated by Student's *t*-test (P value <0.05, P value <0.01, P value <0.001).

Results

Knockout of Notch1 promotes tumor growth of breast cancer by activating the PI3K-AKT pathway

Mice with Lyz2CRE Notch1 +/+ or Lyz2CRE Notch1 flox/flox genotype were generated by inserting 2 LoxP sequences into the 2 ends of the Notch1 gene in mouse

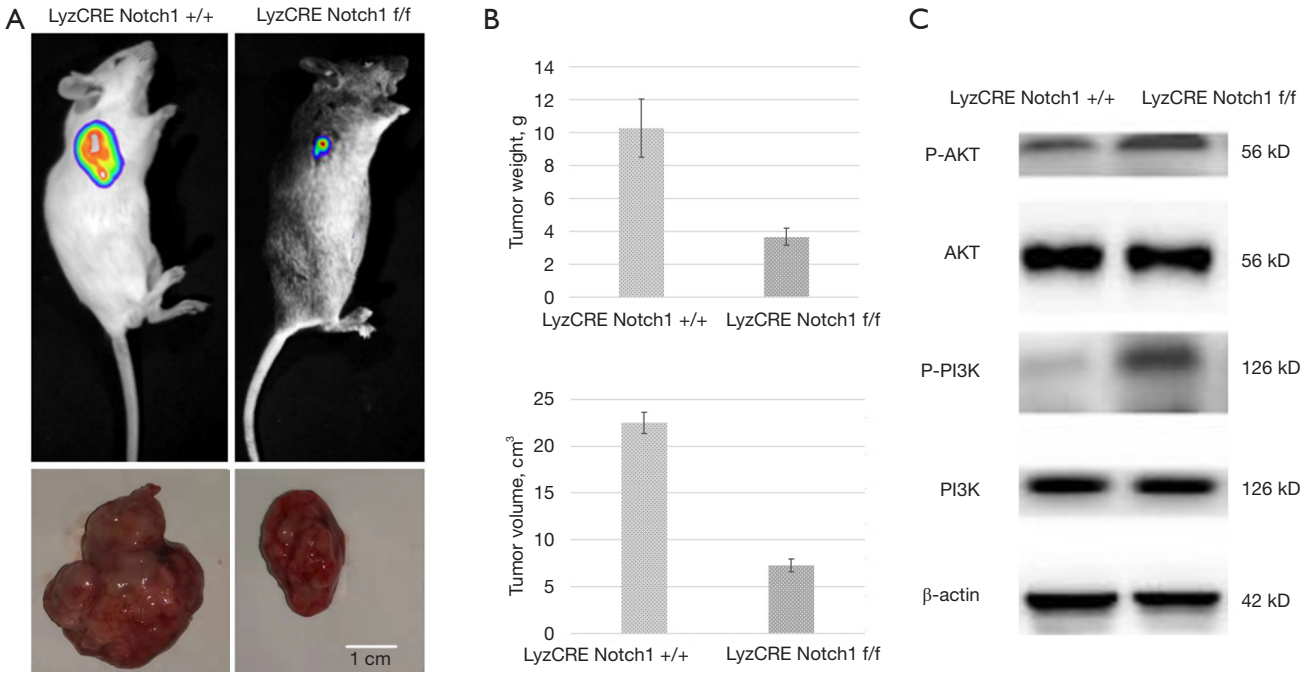

Figure 1 Notch1 knockout in mice promoted breast cancer cell proliferation. (A) The tumor growth of Notch1 knockout mice significantly increased compared with the control group. (B) The data of tumor weight and tumor volume of Notch1 knockout mice and the control group. (C) Notch1 knockout in mice activated the PI3K-AKT pathway.

embryonic stem cells by gene targeting technology. Then, breast cancer FCY20201MCF-7 cells (5×10^6) were inoculated subcutaneously in the lower backs of control and experimental mice with Lyz2CRE Notch1 +/+ or Lyz2CRE Notch1 flox/flox genotype, respectively. These mice had consistent growth conditions and weight. The results demonstrated that Notch1 knockout mice had larger tumors than the control group. The tumor volume was calculated according to the formula: volume = L × S2 × 0.51 by a vernier caliper measurement. The tumor volumes of Notch1 knockout mice were significantly larger than those of the control mice (*Figure 1A*) by about 3.16 times (22.62 *vs.* 7.57 cm^3) (*Figure 1B*). An electronic scale was used to weigh these mice. The tumor weight of Notch1 knockout mice was about 2.15 times the tumor weight of the control mice (10.01 *vs.* 3.40 g) (*Figure 1B*).

The PI3K-AKT-mTOR signal transduction pathway has been become an important research hotspot in the medical field because of its functions in regulating various physiological processes, including proliferation, growth, and survival, among others (13). This prompted us to investigate whether the PI3K-AKT-mTOR signal transduction pathway might contribute to the larger tumor size in Notch1 knockout mice compared with the control group. Therefore, we detected the expression level of PI3K and AKT by western blot. The results are shown in *Figure 1C*. The expression level of phosphorylated AKT (p-AKT) and phosphorylated PI3K (p-PI3K) significantly increased, while the expression of PI3K and AKT did not, resulting in the activation of the PI3K-AKT-mTOR signal pathway. These results indicated that blocking the Notch signal transduction pathway promoted tumor growth in breast cancer by activating the PI3K-AKT signal transduction pathway.

Arginase-1 expression increased by the PI3K-AKT pathway

The above results showed that Notch1 knockout in mice resulted in larger tumors than those in the control group. As shown in previous reports, the Notch signal transduction pathway promotes tumor growth by influencing the tumor microenvironment, especially the expression of inflammatory factors. According to our study, the expression level of arginase-1 increased significantly in Notch1 knockout mice as determined by

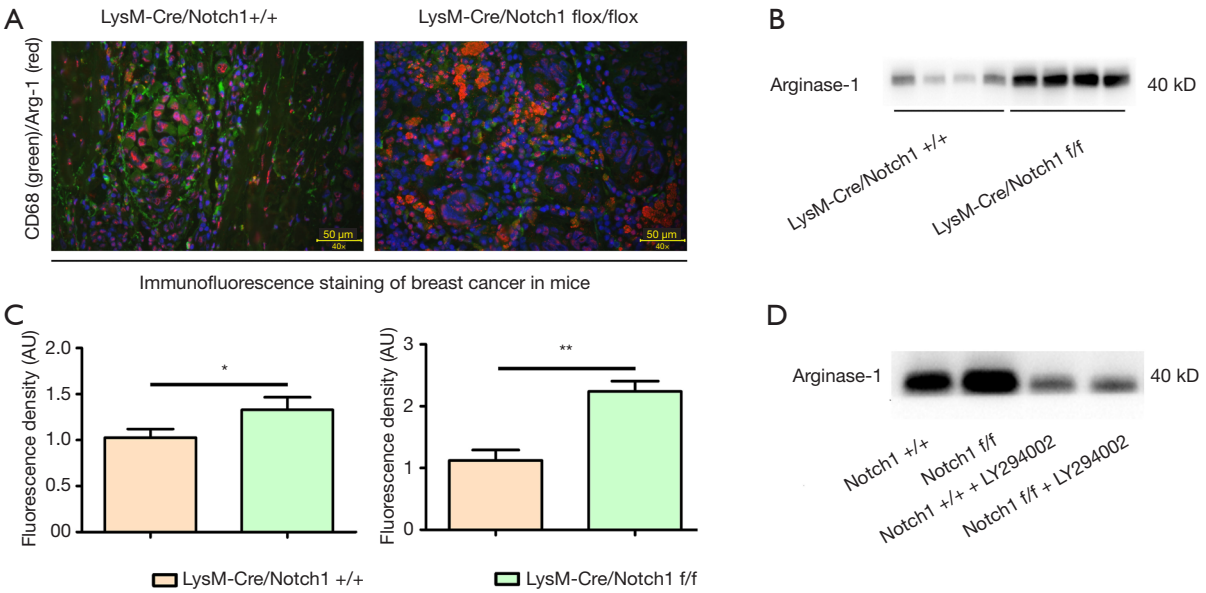

Figure 2 Arginase-1 expression significantly increased, dependent on the PI3K-AKT pathway. (A) Arginase-1 protein expression in breast cancer increased, as determined by immunofluorescence staining. (B) Arginase-1 protein expression in breast cancer increased, as determined by western blot. (C) The data of relative fluorescence density (as in A) and tumor-tissue homogenate levels (as in B). (D) Increased arginase-1 expression could be recovered by administration of LY294002, an inhibitor of PI3K. *, P<0.05; **, P<0.01 and the bar indicated 1 cm.

both immunofluorescence staining and western blot. For immunofluorescence staining, the tumor tissue was fixed by paraformaldehyde, dehydrated by sucrose, frozen, and embedded in OCT compound, then stained by antibodies. Cluster of differentiation 68 (CD68), fused with green fluorescent protein (GFP), was used as a cell marker of TAMs. Arginase-1, fused with red fluorescent protein (RFP), overlapped with CD68 and exhibited stronger fluorescence in the tumor tissue of Notch1 knockout mice compared with the control group (*Figure 2A*). The western blot results showed the same findings (*Figure 2B*). Further, the fluorescence density of RFP and the gray scale of Western blot were quantified and statistically analyzed, in which the results showed that Arginase-1 protein content was almost twice in Notch1 knockout mice that in the control group (*Figure 2C*). Considering that the PI3K/AKT signaling pathway was activated in Notch1 knockout mice, we investigated whether the increased expression of Arginase-1 was due to the activation of the PI3K-AKT pathway. As shown in *Figure 2D*, the expression level of arginase-1 increased in Notch1 knockout mice, which was blocked by feeding mice with LY294002, an inhibitor of PI3K. These results demonstrated that Notch1 knockout promoted the expression of arginase-1 by the PI3K-AKT pathway.

IL-3 and IL-4 expression increased in serum

In recent years, a large number of reports have shown that the tumor microenvironment plays an important role in the development of breast cancer, and TAMs are widely involved in angiogenesis, tumor growth, and metastasis. Under the influence of the local microenvironment, TAMs differentiate into 2 distinct types, namely classically activated macrophages (M1 type) and alternatively activated macrophages (M2 type). It was reported that M2 macrophages played a negative role in inflammatory responses, and may function in the immune escape of tumor cells (14). In this study, the expression level of arginase-1, the M2-type cytokine, increased significantly. This result indicated that TAMs in Notch1 knockout mice polarized into the M2 type, which may be the cause of tumor proliferation in breast cancer. In addition, IL-3 and IL-4 are also M2-type cytokines whose protein levels in serum were examined by ELISA. As shown in *Figure 3A*, IL-3 and IL-4 protein content was higher in the serum of Notch1 knockout mice than that in the control group (*Figure 3B*).

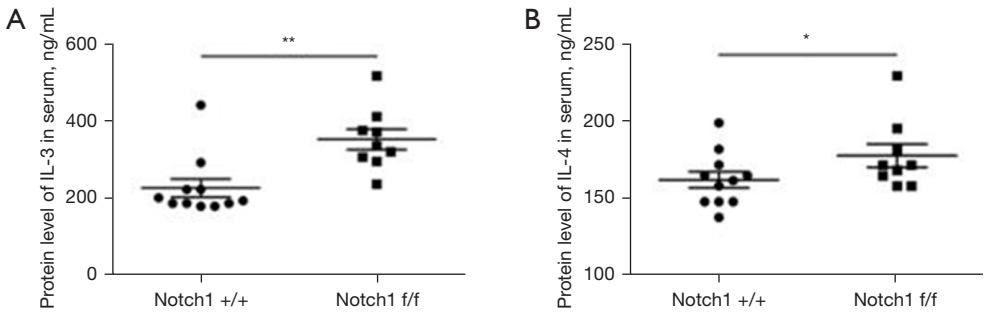

Figure 3 The protein content of IL-3 (A) and IL-4 (B) in the serum of Notch1 knockout mice significantly increased. *, P<0.05; **, P<0.01.

These results demonstrated that Notch1 knockout resulted in the polarization of TAMs towards the M2-type phenotype to cause tumor proliferation in breast cancer.

Discussion

A large number of studies have shown that the tumor microenvironment plays an important role in tumorigenesis, cell proliferation, and immune escape, in which TAMs also play an important role. Macrophages differentiate from monocytes (15-17). Monocytes are derived from bone marrow stem cells, which after being released into peripheral blood, are further differentiated into tissue-specific macrophages. TAMs are tissue-specific macrophages which are located in tumor tissue. Macrophages, as an important component of the innate immune system, function in phagocytosis, antigen presentation, and the secretion of various cytokines, and play a necessary role in the inflammation process, defense, repair, and metabolism. TAMs are induced by the influence of the local microenvironment to polarize into 2 different types, namely classically activated macrophages (M1 type) and alternatively activated macrophages (M2 type). M1 macrophages promote inflammation by being mainly involved in the Th1-type immune response (18). However, M2-type TAMs play a negative role in inflammation through the Th2-type immune response (14). According to previous studies, researchers have discovered highly infiltrated M2-type TAMs in various malignant tumors, including lung cancer (19) and liver cancer (20), among others (21), which indicates poor prognosis. In this study, our results demonstrated that knockout of Notch1 in mice promoted the growth of breast cancer (*Figure 1A*,*1B*), similar to previous study in osteosarcoma (22). Further results proved that increased tumor proliferation resulted from not only the activation of the PI3K-AKT pathway (*Figure 1C*), but also the polarization of TAMs towards the M2-type phenotype (*Figures 2*,*3*). A large number of studies demonstrated that the PI3K-AKT pathway could promote proliferation in various malignant tumors, including breast cancer (23-25). This result seems to explain the increased tumor proliferation after knockout of Notch1. In addition, the expression of M2-type inflammatory factors, such as arginase-1, IL-3, and IL-4, significantly increased (*Figures 2*,*3*). These findings demonstrated that M2-type TAMs may be involved in the immune escape of tumor cells in various malignant tumors, indicating that the polarization of TAMs towards the M2-type phenotype in Notch1 knockout mice might contribute to the increased tumor growth. On the basis of these results, we hypothesized that Notch1 knockout in mice may promote the distant metastasis of breast cancer. Meanwhile, it was proven that the increased expression of arginase-1 was dependent on the activation of the PI3K-AKT pathway, indicating the intrinsic link between these 2 signal pathways. However, the underlying molecular mechanism needs further study.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-1041/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-1041/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.

amegroups.com/article/view/10.21037/atm-22-1041/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (No. IACUC-4th Hos Hebmu-20180212) granted by the experimental animal ethics committee of the Fourth Hospital of Hebei Medical University, in compliance with the national guidelines for the care and use of animals.

References

1. Akarolo-Anthony SN, Ogundiran TO, Adebamowo CA. Emerging breast cancer epidemic: evidence from Africa. Breast Cancer Res 2010;12 Suppl 4:S8.
2. Tan AR, Im SA, Mattar A, et al. Fixed-dose combination of pertuzumab and trastuzumab for subcutaneous injection plus chemotherapy in HER2-positive early breast cancer (FeDeriCa): a randomised, open-label, multicentre, non-inferiority, phase 3 study. Lancet Oncol 2021;22:85-97.
3. Whang J, Steinkellner AR, Borne JM, et al. Quantifying the relative value of drug therapy options in HER2+, ER-/PR- breast cancer. J Clin Oncol 2015;33:6602.
4. Leaf S, Carlsen L, El-Deiry WS. Opposing effects of BRCA1 mRNA expression on patient survival in breast and colorectal cancer and variations among African American, Asian, and younger patients. Oncotarget 2021;12:1992-2005.
5. Franklin RA, Liao W, Sarkar A, et al. The cellular and molecular origin of tumor-associated macrophages. Science 2014;344:921-5.
6. Shen Q, Cohen B, Zheng W, et al. Notch Shapes the Innate Immunophenotype in Breast Cancer. Cancer Discov 2017;7:1320-35.
7. Sousa S, Brion R, Lintunen M, et al. Human breast cancer cells educate macrophages toward the M2 activation status. Breast Cancer Res 2015;17:101.
8. Movahedi K, Laoui D, Gysemans C, et al. Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. Cancer Res 2010;70:5728-39.
9. Kovaleva OV, Samoilova DV, Shitova MS, et al. Tumor Associated Macrophages in Kidney Cancer. Anal Cell Pathol (Amst) 2016;2016:9307549.
10. Xu J, Chi F, Guo T, et al. NOTCH reprograms mitochondrial metabolism for proinflammatory macrophage activation. J Clin Invest 2015;125:1579-90.
11. Xu H, Zhu J, Smith S, et al. Notch-RBP-J signaling regulates the transcription factor IRF8 to promote inflammatory macrophage polarization. Nat Immunol 2012;13:642-50.
12. Purow B. Notch inhibition as a promising new approach to cancer therapy. Adv Exp Med Biol 2012;727:305-19.
13. Makwana V, Rudrawar S, Anoopkumar-Dukie S. Signalling transduction of O-GlcNAcylation and PI3K/AKT/mTOR-axis in prostate cancer. Biochim Biophys Acta Mol Basis Dis 2021;1867:166129.
14. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 2009;27:451-83.
15. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. Immunity 2010;32:593-604.
16. Gunaydin G. CAFs Interacting With TAMs in Tumor Microenvironment to Enhance Tumorigenesis and Immune Evasion. Front Oncol 2021;11:668349.
17. Wang S, Ma F, Feng Y, et al. Role of exosomal miR21 in the tumor microenvironment and osteosarcoma tumorigenesis and progression (Review). Int J Oncol 2020;56:1055-63.
18. Sun R, Abraham C. IL23 Promotes Antimicrobial Pathways in Human Macrophages, Which Are Reduced With the IBD-Protective IL23R R381Q Variant. Cell Mol Gastroenterol Hepatol 2020;10:673-97.
19. Lu CS, Shiau AL, Su BH, et al. Oct4 promotes M2 macrophage polarization through upregulation of macrophage colony-stimulating factor in lung cancer. J Hematol Oncol 2020;13:62.
20. Sun S, Cui Y, Ren K, et al. 8-bromo-7-methoxychrysin Reversed M2 Polarization of Tumor-associated Macrophages Induced by Liver Cancer Stem-like Cells. Anticancer Agents Med Chem 2017;17:286-93.
21. Farajzadeh Valilou S, Keshavarz-Fathi M, Silvestris N,

et al. The role of inflammatory cytokines and tumor associated macrophages (TAMs) in microenvironment of pancreatic cancer. Cytokine Growth Factor Rev 2018;39:46-61.
22. Ren S, Zhang X, Hu Y, et al. Blocking the Notch signal transduction pathway promotes tumor growth in osteosarcoma by affecting polarization of TAM to M2 phenotype. Ann Transl Med 2020;8:1057.
23. Atif F, Yousuf S, Stein DG. Anti-tumor effects of progesterone in human glioblastoma multiforme: role of PI3K/Akt/mTOR signaling. J Steroid Biochem Mol Biol 2015;146:62-73.
24. Wu YR, Qi HJ, Deng DF, et al. MicroRNA-21 promotes cell proliferation, migration, and resistance to apoptosis through PTEN/PI3K/AKT signaling pathway in esophageal cancer. Tumour Biol 2016;37:12061-70.
25. Liu Y, Pan YF, Xue YQ, et al. uPAR promotes tumor-like biologic behaviors of fibroblast-like synoviocytes through PI3K/Akt signaling pathway in patients with rheumatoid arthritis. Cell Mol Immunol 2018;15:171-81.

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