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PNMA5 Promotes Radiotherapy Resistance in Colorectal Cancer by Regulating Angiogenic Factor Secretion and Apoptosis

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Abstract: Colorectal cancer (CRC) is a frequent cancer in the digestive tract, and its occurrence and death rates are high all over the world, so it is a big health issue; PNMA5 can promote radiotherapy resistance in CRC, and it does so by regulating the release of angiogenic factors and cell death; this experiment used both cell and animal CRC models to verify the role of PNMA5. In this study, we generated PNMA5 models where we either overexpress or knock down genes in HT-29 cells. Six groups were included: HT-29 control, HT-29+RT, HT-29-NC, HT-29-si-PNMA5, HT-29-OE-PNMA5, and HT-29-OE-PNMA5+RT. TUNEL assay measures the level of apoptosis and apoptosis by flow cytometry. For paracrine proangiogenic capacity, we performed HUVEC tube formation assays and ELISA. In vivo. Tumor cells were put into nude mice, and these mice were treated with radiation; the purpose was to check how sensitive the tumors were to radiotherapy and to measure the density of micro vessels (MVD). To figure out which pathways PNMA5 controls downstream, we ran transcriptomic sequencing (RNA-seq) followed by GSEA. The results show that in cell experiments, after receiving 18 Gy radiation, HT29 cells with PNMA5 overexpression grew more quickly and showed a lower death rate; Western blot analyses revealed that BCL-2 expression was raised and BAX activation was inhibited by PNMA5; and ELISA together with tube formation assays confirmed that PNMA5 enhanced the secretion of VEGF, FGF-1, and TGF- β 1, which then improved the tube-formation ability of blood vessel endothelial cells. Inside a living organism, the growth rate and micro vessel density (MVD) of tumors in the OE-PNMA5 group got significantly bigger than controls when exposed to radiotherapy. Transcriptomic sequencing further revealed that, PNMA5 does not directly activate the transcription of the VEGF gene. Instead, it activates STAT1 and IRF7, maintaining the interferon response and chemokine-related signals. This, in turn, alters the secretions of tumor cells, and through paracrine pathways, indirectly promotes angiogenesis, thereby causing radiotherapy resistance. Therefore, we can conclude that PNMA5 mediates

radiotherapy resistance in colorectal cancer in two aspects. One is its synergistic effect with the intrinsic apoptosis inhibition of cells, and the other is its alteration of tumor secreted substances, which promotes angiogenesis through paracrine pathways; PNMA5 activates STAT1 and IRF7, thereby influencing the interconnection of the microenvironment and providing a new perspective for radiotherapy resistance.

Keywords: PNMA5; CRC; Radio resistance; Angiogenesis; the VEGF pathway.

1. INTRODUCTION

CRC is very common around the world—a malignant tumor that starts in the digestive system. In the U.S., it's the second largest cancer killer, and for adults under 50, it's number one.¹ When you're dealing with locally advanced rectal cancer, neoadjuvant chemoradiotherapy (nCRT) is often used first and has become the standard treatment regimen, aiming to shrink tumors, improve radical resection rates, and increase sphincter-preservation rates. However, radio resistance remains a bottleneck in clinical therapy, with approximately 40% to 60% of patients exhibiting insensitivity to radiotherapy, which consequently raises the chance of the tumor coming back in the same place². Identifying the mechanisms and sensitizing targets for CRC radio resistance is of high clinical relevance.

Radio resistance is a complex process driven co-operatively by intrinsic tumor defenses and the extrinsic microenvironment. The TME also plays a significant role, alongside cellular-intrinsic factors like enhanced DNA damage repair and autophagy activation. VEGF-mediated abnormal vascular dilation and dysfunction lead to a severely hypoxic intratumoral microenvironment, which significantly impairs the lethal effects of radiation-induced oxygen free radicals³⁻⁵. Anti- VEGF agents are part of standard care, yet tumors frequently develop resistance by turning on bypass pathways. Therefore, identifying upstream master switches that link vascular abnormalities with radio resistance has become a central focus of current research.

At least 15 PNMA family proteins exist, with functions that differ by tumor type⁶⁻⁸. PNMA1 and PNMA2 have opposing roles in apoptosis⁹⁻¹¹, while PNMA4 (MOAP1) is associated with chemosensitivity¹²⁻¹⁴, and PNMA4 (MOAP1) relates to chemosensitivity¹⁵⁻¹⁷. PNMA5 is notable for its unique sequence and distribution. Although it promotes malignancy in ovarian, liver, and lung cancers¹⁸⁻²⁰, no data exist on its involvement in CRC or radiation response.

Our research group previously found through bioinformatic mining (TCGA and UALCAN databases) that PNMA5 levels are way higher in CRC tissues than normal, and its expression levels increase with clinical stage progression, suggesting that PNMA5 may be a potential oncogene in CRC. Preliminary experiments confirmed that intervening in PNMA5 expression significantly affects the proliferation and apoptosis levels of HT-29 cells and influences tumor

growth in nude mouse models. Combined with clinical challenges, we hypothesise that PNMA5 could be important for CRC radiotherapy resistance.

Based on bioinformatics analysis and previous results, in both *in vitro* and *in vivo* experiments, it was verified that PNMA5 not only affects the proliferation and apoptosis of colorectal cancer cells, but also regulates micro vessel density and the secretion of pro-angiogenic factors, participating in radiotherapy resistance; the transcriptome sequencing results suggest that PNMA5 does not directly activate the classical VEGF pathway at the transcriptional level, but significantly enriches the chronic interferon response and chemokine signaling network centered on STAT1 and IRF7; under the effect of radiotherapy, tumor cells activate such non-classical signals as STAT1/IRF7, thereby changing the composition of cell secretions and indirectly stimulating angiogenesis through paracrine; thus, a hypothesis is formed: as an upstream key molecule, PNMA5 changes the secretome by non-classical activation of STAT1/IRF7, and then indirectly promotes angiogenesis through the interaction between immunity and blood vessels, ultimately leading to resistance of colorectal cancer to radiotherapy.

Stable cell lines overexpressing and knocking down PNMA5 were constructed, and PNMA5's effect on proliferation, apoptosis and pro-angiogenic ability of CRC cells. Based on the results of transcriptome sequencing, the downstream non-classical mechanisms were elucidated. At the same time, PNMA5 was explored as the upstream key molecule that regulates the apoptosis balance within the cell and the changes in excretions, thereby participating in the regulation of radiotherapy sensitivity. These findings can provide a new perspective on the microenvironment of radio resistance and also offer theoretical basis and possible targets for individualized radiotherapy and anti-angiogenic combined therapy.

2. MATERIALS AND METHODS

2.1 Cell

All stable transfection cell lines and the original HT-29 cells were kept in DMEM high-glucose media. The volume fraction of fetal bovine serum in this medium was 10%, and the proportion of the double antiserum (penicillin-streptomycin mixture) was 1%. The culture environment was set at 37°C with a CO₂ concentration of 5% in a saturated humidity incubator. When the cell proliferation was vigorous, passages were performed as needed, usually once every 2 to 3 days.

2.2 Lentiviral Transfection and Selection of Stable Cell Lines

We got lentiviral constructs for PNMA5 overexpression (OE-PNMA5), shRNA-mediated knockdown (si-PNMA5), and an empty vector negative control (NC) from Inner Mongolia Hongke Biotechnology Co., Ltd., which also handled the packaging. HT29 cells got infected with lentivirus at an MOI of about 10–20; we added polybrene (8 µg/mL) during viral transduction to improve infection efficiency. After that, to obtain stable lines, we chose

transduced cells and treated them with 2 µg/mL puromycin for two weeks. Knockdown and overexpression efficiencies were then checked by Western blotting.

2.3 Radiotherapy Treatment

We used an X-ray irradiator to deliver local radiation to cells and tumor-bearing nude mice at 2 Gy per minute. Each fraction was 6 Gy, given once every other day for three fractions, for a total cumulative dose of 18 Gy. The non-irradiated groups were given sham irradiation under the same conditions.

2.4 TUNEL Apoptosis Assay

The main steps of the TUNEL apoptosis assay can be divided into: 1. Sample preparation and pretreatment: adherent cells, suspension cells, paraffin sections, and frozen sections; 2. Fixation and permeabilization; 3. TUNEL labeling reaction: equilibration, preparation of the detection solution, and incubation; 4. Washing and counterstaining; 5. Mounting and observation.

2.5 FCM (flow cytometry)

An Annexin V-FITC/PI apoptosis detection kit was used to assess apoptosis (MultiSciences, AP101). Briefly, harvested cells were resuspended in binding buffer after being thrice cleaned with ice-cold PBS, followed by incubation for 15 minutes at room temperature in the dark with 5 µL of Annexin V-FITC and 5 µL of propidium iodide (PI), with 5 µL of Annexin V-FITC and 5 µL of propidium iodide (PI). Subsequently, the proportion of Annexin V-positive cells were quantified using FlowJo software (v10), and the samples were analyzed using a BD Cascante II flow cytometer. All measurements were done within an hour of collecting the samples.

2.6 In Vitro Tube Formation Assay

We switched each group of cells to serum-free medium and left them to grow for 24 hours. Then we collected the conditioned medium (CM) and spun it at 1000 rpm for 5 minutes to get rid of cell debris. For the tube formation assay, we coated precooled 96-well plates with 50 µL of Matrigel (ABW, 0827035) per well, and after 30 minutes of incubation at 37°C, the Matrigel was permitted to solidify, the Matrigel was permitted to solidify. The gel was inoculated with HUVECs at a 2×10^2 cell density per well, and 100 µL conditioned media (CM) from each group was subsequently added. We incubated the cells at 37 °C for 6–8 hours. Then we took photos of tube formation with an inverted microscope. In each well, we randomly chose five fields. We used ImageJ to count the complete capillary-like tubes.

2.7 ELISA

We bought commercial ELISA kits. We used these kits to measure the levels of VEGF, FGF-1, and TGF-β1 in the cell culture supernatants from each group. We also measured the levels of TGF-β1 and FGF in the serum of nude mice. The kit catalog numbers are VEGF (EH0327), FGF-1 (EH0501), and TGF-β1: Cat. No. EH0287 (Wuhan Cusabio Biotech Co.,

Ltd.). The manufacturer's instructions were closely followed when performing the procedures. The optical density (OD) of each well was tested at a 450 nm wavelength after the microplate reader was adjusted to zero using the blank wells. Example concentrations for each group's values were obtained from the corresponding standard curves.

2.8 Western Blotting

We lysed both the cells and tumor tissues with RIPA lysis buffer (Bioreligy, BN25001) spiked with inhibitors for both proteases and phosphatases. We measured protein concentrations with the BCA method (UtiBody, SL201). Proteins (30 µg per lane) were divided by SDS-PAGE (Boster, AR0138) and subsequently deposited onto Millipore. After blocking membranes with 5% non-fat milk for one hour at room temperature, primary antibodies (all 1:1,000; Proteintech) against PNMA5, BAX, BCL2, VEGFA, VEGFR2, and β-actin were incubated overnight at 4°C. After washing, the samples were incubated with HRP-conjugated secondary antibodies (1:5,000) for 1 hour at room temperature. Bands were developed with ECL (Bioreligy, BN16009) and quantified densitometrically (ImageJ).

2.9 Animal Experiments

We obtained ethics approval (2025GLLH0253) from the Affiliated Tumor Hospital of Inner Mongolia Medical University's Experimental Animal Ethics Committee and followed its institutional guidelines for animal care. Beijing SPF Biotechnology Co., Ltd. sold four- to five-week-old C naked mice, and they were grown in SPF conditions. We established subcutaneous tumors by injecting 5×10^6 HT-29 cells temporarily halted in 100 µL PBS into the correct side. Six groups of mice (n = 6 each) were created as follows: HT-29, HT-29+RT, HT-29-NC, HT-29-si-PNMA5, HT-29-OE-PNMA5, and HT-29-OE-PNMA5+RT. After tumors reached ~100 mm³, we monitored growth every three days by measuring L and W with callipers and computed the volume using $\frac{1}{2} \times L \times W^2$. We monitored body weight throughout the study. Mice were anesthetized via intraperitoneal injection of sodium pentobarbital (50 mg/kg) prior to the treatment, and all efforts were made to minimize animal suffering. At the endpoint, we euthanised mice by cervical dislocation. After collecting retroorbital blood and centrifuging it for 15 minutes at 3,000 rpm, the serum was saved for ELISA.

2.10 IHC and TUNEL

The tumor sections were cut into 4-micron-thick slices. After dewaxing and rehydration, the slices were incubated overnight at 4°C with anti-Ki67 and anti-CD31 antibodies after being blocked with 5% bovine serum albumin (BSA) for antigen retrieval. After washing, the sections were treated for one hour at room temperature using secondary antibodies conjugated with HRP. The immune reaction was detected using the DAB kit (Zhongshan Jinqiao Biotechnology), and hematoxylin was used to stain the cell nuclei, which were then examined beneath a slide. The number of apoptotic cells was detected by TUNEL staining (Servicebio, G1504) of the tumor sections.

2.11 Transcriptomic Sequencing and Bioinformatics Analysis

The HT-29, HT-29+ radiotherapy, and HT-29-OE-PNMA5 cell groups were respectively inoculated into the transplanted tumors formed in the subcutaneous areas of nude mice. The RNA extraction from the tumors was quality-checked ($RIN \geq 7.0$) using the Agilent 2100 equipment and sequenced (by Ouyi Biomedical Technology Company). The clean reads were aligned with GRCh38 using HISAT2. DESeq2 identified DEGs ($FDR < 0.05$, $|\log_2FC| \geq 1$). Enrichment pathways were analyzed using KEGG (cluster Profiler) and GSEA (v4.3, for Hallmark genes).

2.12 Statistical Analysis

All experiments were repeated three times, and data are shown as mean $\pm$ SD. We did the statistical analysis with GraphPad Prism (v9.0). For more than two groups, one-way ANOVA and Tukey's test were used, and for two groups, we used Student's t-test.

3 RESULTS

3.1 Overexpression of PNMA5 and clinical stage.

Compared with the normal tissues adjacent to the cancer, PNMA5 expression in CRC was significantly upregulated (TCGA, $P < 0.001$). This result persisted throughout all stages (I to IV) and showed little difference between them. High PNMA5 predicted poor survival. PNMA5 appears to function as an oncogene in CRC and may contribute to disease progression.

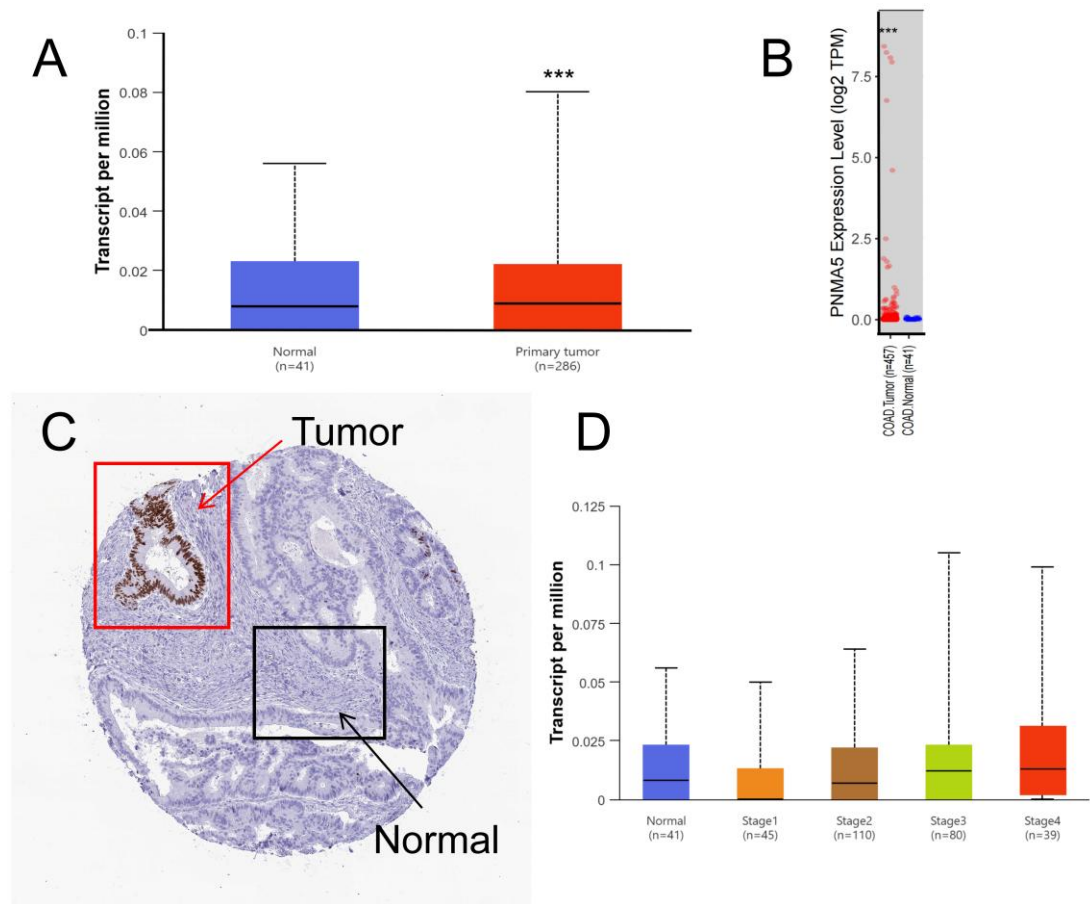


Figure 1. PNMA5 expression in CRC and its correlation with the clinical prognosis

(A) UALCAN-based examination of TCGA data showing differential PNMA5 mRNA expression between colorectal cancer (COAD) and normal tissues. (B) Validation of PNMA5 upregulation in COAD using the TIMER2.0 database ($P < 0.001$, tumor vs. normal). (C) The Human Protein Atlas (HPA) provides representative immunohistochemistry (IHC) pictures of PNMA5 protein expression in normal colon and colorectal cancer tissues, showing strong cytoplasmic staining in tumor cells (Antibody ID: HPA052473). (D) PNMA5 mRNA expression across clinical stages I–IV based on UALCAN analysis. "n" indicates sample size per group. The KEGG pathway map was reproduced or adapted with permission from Kanehisa Laboratories.

3.2 PNMA5 Inhibits Apoptosis and Radiation Resistance in CRC

The TUNEL staining method was used to detect the level of apoptosis in HT-29 cells in each group. Under non-irradiated conditions (0 Gy), PNMA5 knockdown (HT-29-si-PNMA5) significantly induction of apoptosis in HT-29 cells compared with the negative control (HT-29-NC), whereas PNMA5 overexpression (HT-29-OE-PNMA5) markedly inhibit cell apoptosis (Fig. 2A, B). Following radiation therapy (18 Gy), the TUNEL-positive rate in the HT-29+RT group was significantly higher than that in the HT-29 group ($P < 0.01$), demonstrating that radiation therapy can effectively induce apoptosis in colorectal cancer cells. The TUNEL-positive rate in the HT-29-OE-PNMA5+RT group was significantly lower than that in the HT-29+RT group ($P < 0.001$), suggesting that PNMA5 overexpression partially confers resistance to radiotherapy-induced apoptosis, indicating that PNMA5 may mediate radiotherapy resistance in colorectal cancer by inhibiting apoptosis.

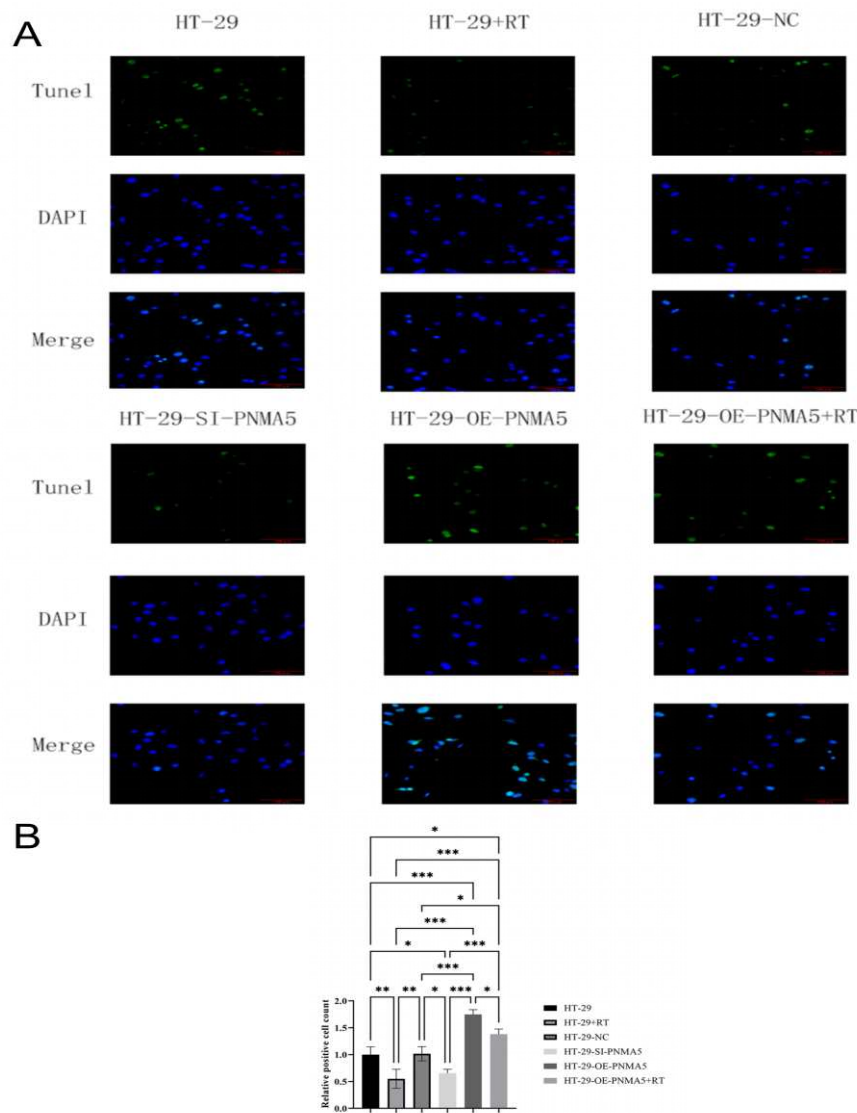


Figure 2. Assessment of the Effects of PNMA5 on Apoptosis and Radiation Sensitivity in HT-29 Cells Using the TUNEL Assay.

(A) TUNEL Staining showing apoptosis level across experimental groups. Images compare cells under non-irradiated (0 Gy) conditions—HT-29, HT-29-NC, HT-29-si-PNMA5, and HT-29-OE-PNMA5—and after 18 Gy irradiation (HT-29-Vector+RT, HT-29-OE-PNMA5+RT). TUNEL-positive (apoptotic) cells stain green; nuclei are counterstained with DAPI (blue). Scale bar, 100 μ m. (B) Quantitative statistical analysis of the TUNEL-positive across the groups. The results demonstrate that PNMA5 knockdown induce apoptosis, whereas PNMA5 overexpression significantly counteracts apoptosis induced by radiotherapy. The data are displayed as mean $\pm$ standard deviation (SD) (n = 3). One-way analysis of variance (ANOVA) was used to assess statistical significance. *P < 0.05, **P < 0.01, and ***P < 0.001.

3.3 PNMA5 Inhibits Radiation-Induced Apoptosis and Controls the levels of proteins involved in apoptosis

We used flow cytometry to measure apoptosis in irradiated (18 Gy) HT-29 cells (Fig. 3A, B) to test whether PNMA5-driven radio resistance involves apoptosis blockade. In non-irradiated cells, PNMA5 knockdown slightly raised apoptosis, but overexpression left the baseline largely unchanged. After 18 Gy irradiation, both HT-29 control and NC groups exhibited a marked increase in apoptosis, consistent with the expected cytotoxic effect of radiation. But the OEPNMA5+RT group had significantly fewer apoptotic cells than the radiation alone (HT29+RT) group (Fig. 3B). So PNMA5 clearly dampens the pro- apoptotic effect of radiation.

We then checked the amounts of the apoptosis-inhibiting protein BCL2 and the pro-apoptotic protein BAX by WB (Fig 3C, D). Radiation alone strongly increased BAX expression in HT- 29 cells. However, under the same radiation dose, the OEPNMA5+RT group had lower BAX levels than the radiation-alone group. Meanwhile, PNMA5 overexpression raised basal BCL2 levels and kept them high even after radiation, while knocking down PNMA5 sharply reduced BCL2 expression. By downregulating BAX and upregulating BCL2, PNMA5 protects CRC cells from radiation-induced apoptosis

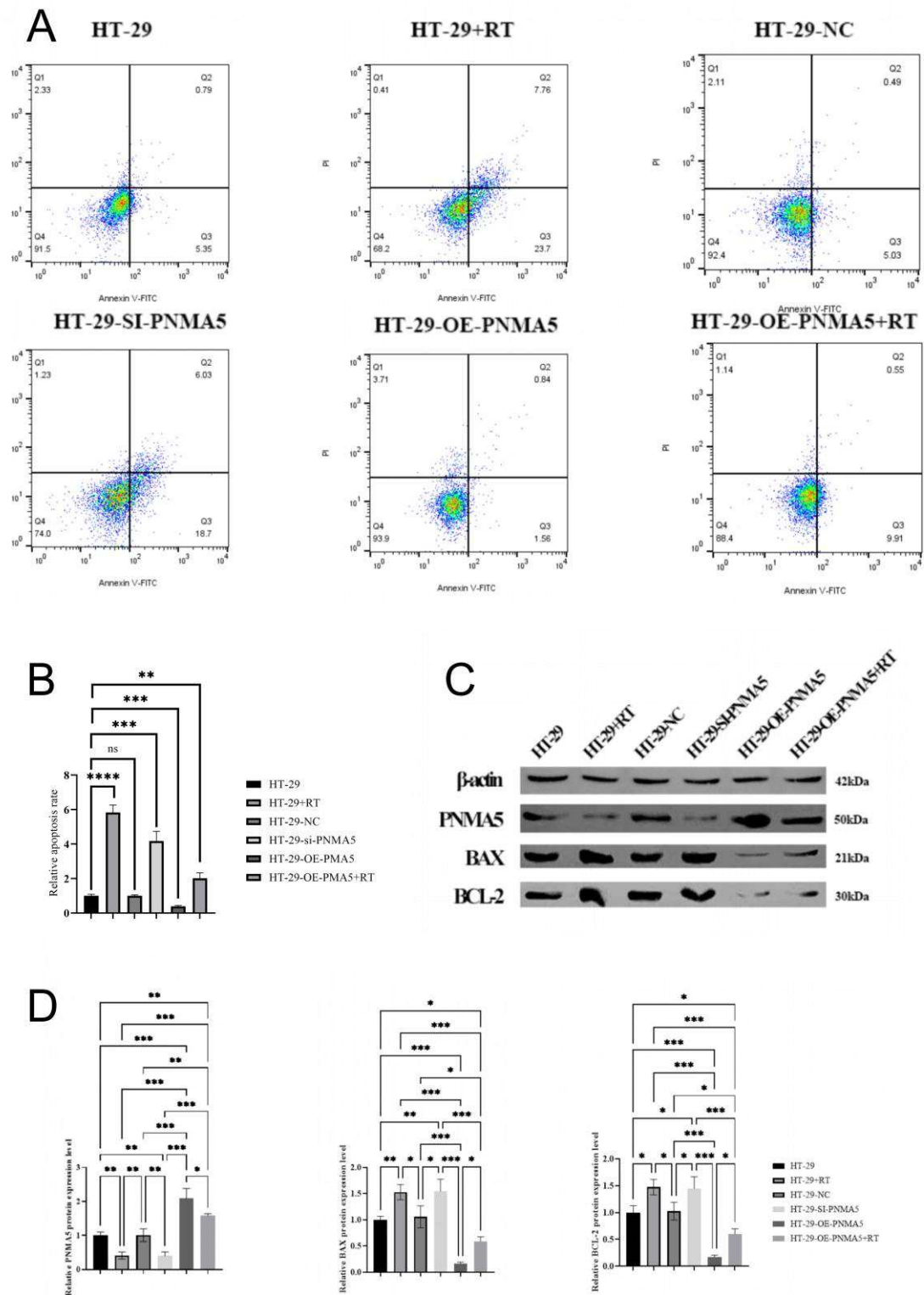


Figure 3. PNMA5 inhibits radiation-induced apoptosis by regulating apoptosis-related proteins.

(A) Analysis using flow cytometry to identify the apoptosis of HT-29 cells in each group under 0 Gy or radiotherapy (RT, 18 Gy) treatments. Lower-left, right, and upper-right (viable cells, early apoptotic cells, and late apoptotic cells) are represented by the quadrants. (B) Quantitative analysis of the total apoptosis rate (early + late apoptosis) for each group. PNMA5 overexpression reduced radiation-induced apoptosis, as shown by WB (C) and densitometric quantification (D) (mean $\pm$ SD, n = 3; *P < 0.001).

3.4 PNMA5 drives angiogenic factor secretion and thus supports vascular tube formation.

To test whether PNMA5 modulates the microenvironment via paracrine signaling, we quantified pro-angiogenic factors in HT-29 conditioned media (Fig. 4A). Without irradiation, OE-PNMA5 elevated VEGF, FGF-1, and TGF- β 1, whereas si-PNMA5 lowered them. After 18 Gy, the radiation-only group showed a cytokine rebound, but OE-PNMA5+RT cells still had significantly higher levels (P < 0.05), indicating that PNMA5 reinforces a pro-angiogenic environment under stress.

HUVEC tube formation assays (Fig. 4B, C) were used to assess the angiogenic activity of secreted factors. Conditioned medium (CM) from OE-PNMA5 cells promoted tube formation—increasing total tube length and branch points—compared with NC-derived CM. This pro-angiogenic effect persisted after irradiation: CM from the OE-PNMA5+RT group still surpassed the radiation-only group in tube formation.

By upregulating the production and paracrine release of pro-angiogenic factors (e.g., VEGF) upon radiation, PNMA5 skews the tumor microenvironment toward angiogenesis—an extrinsic process that likely underpins radio resistance.

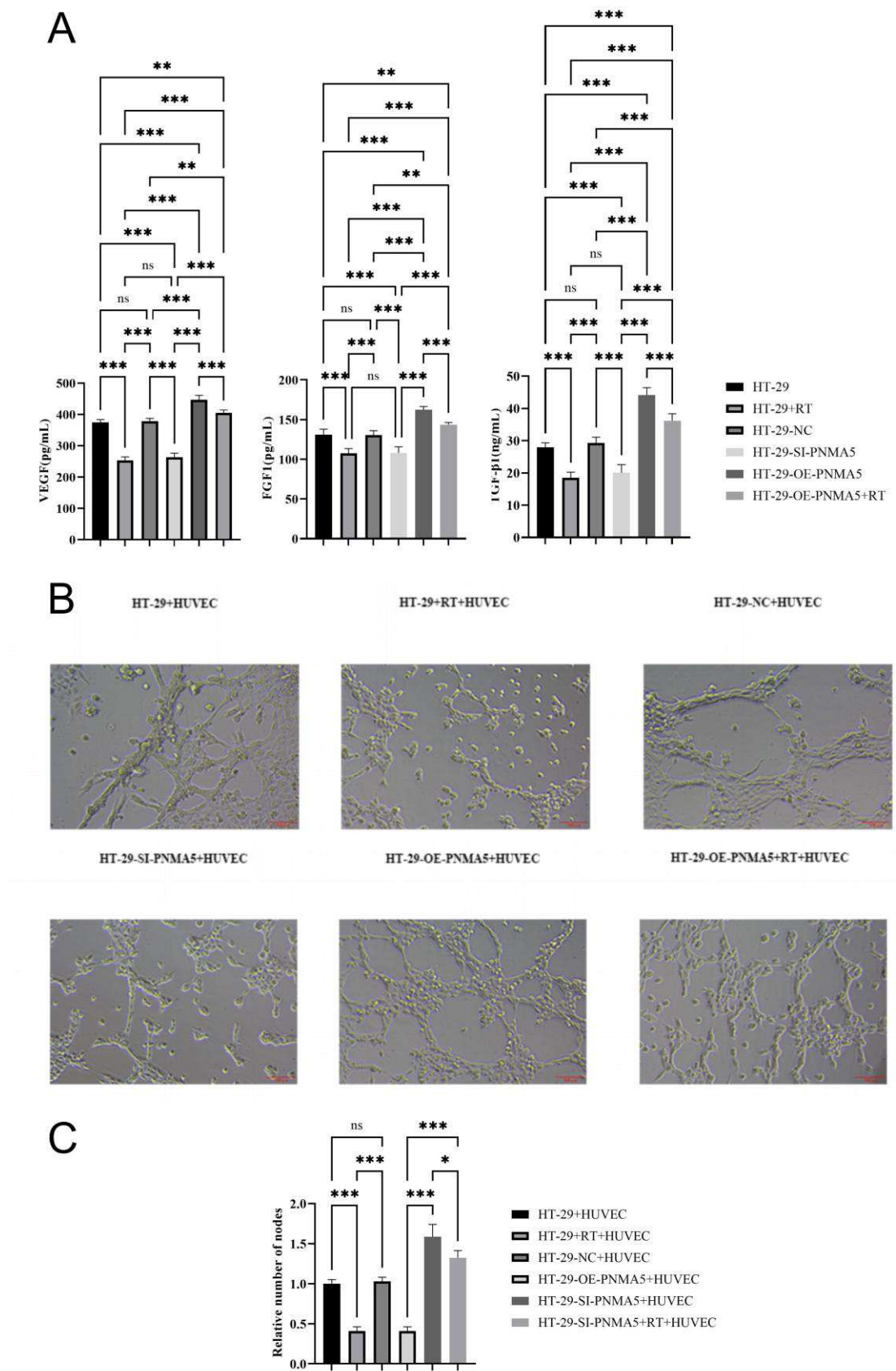


Figure 4. PNMA5 promotes the paracrine secretion of pro-angiogenic factors and enhances endothelial tube formation. (A) ELISA quantification of VEGF, FGF-1, and TGF- β 1 in the culture

supernatants of the indicated HT-29 groups. PNMA5 overexpression substantially increased the secretion of these factors, and this pro-angiogenic capacity was maintained after irradiation (RT). (B) Representative images of the HUVEC tube formation assay performed with conditioned medium (CM) from each group. CM derived from PNMA5-overexpressing cells induced more extensive and intact capillary-like networks. Scale bar, 200 μ m. (C) Quantification of branch points concerning the tube formation test. One-way ANOVA is used to show the data as the mean $\pm$ standard deviation (n =) = 3, $P < 0.05$, * $P < 0.01$, *** $P < 0.001$ **).

3.5 PNMA5 accelerates colorectal cancer growth and reduces radiosensitivity in vivo

With treatments given as described, we made a tumor model under the skin in nude mice; then we tested how PNMA5 affected tumor growth and radiation sensitivity. (Fig. 5A). Analysis of ultimate tumor weights and tumor growth curves revealed that, in the absence of radiation, the OE-PNMA5 group exhibited markedly accelerated tumor growth compared with controls, whereas PNMA5 knockdown significantly suppressed tumor progression. Following irradiation, tumor growth was inhibited across all groups; however, the OE-PNMA5+RT group maintained significantly larger tumor volumes and weights than the radiation-alone group ($P < 0.05$). So PNMA5 clearly counteracts radiation's anti-tumor effect in vivo. Importantly, mouse body weights stayed stable throughout the experiment, with no obvious toxicity (Fig 5B, C).

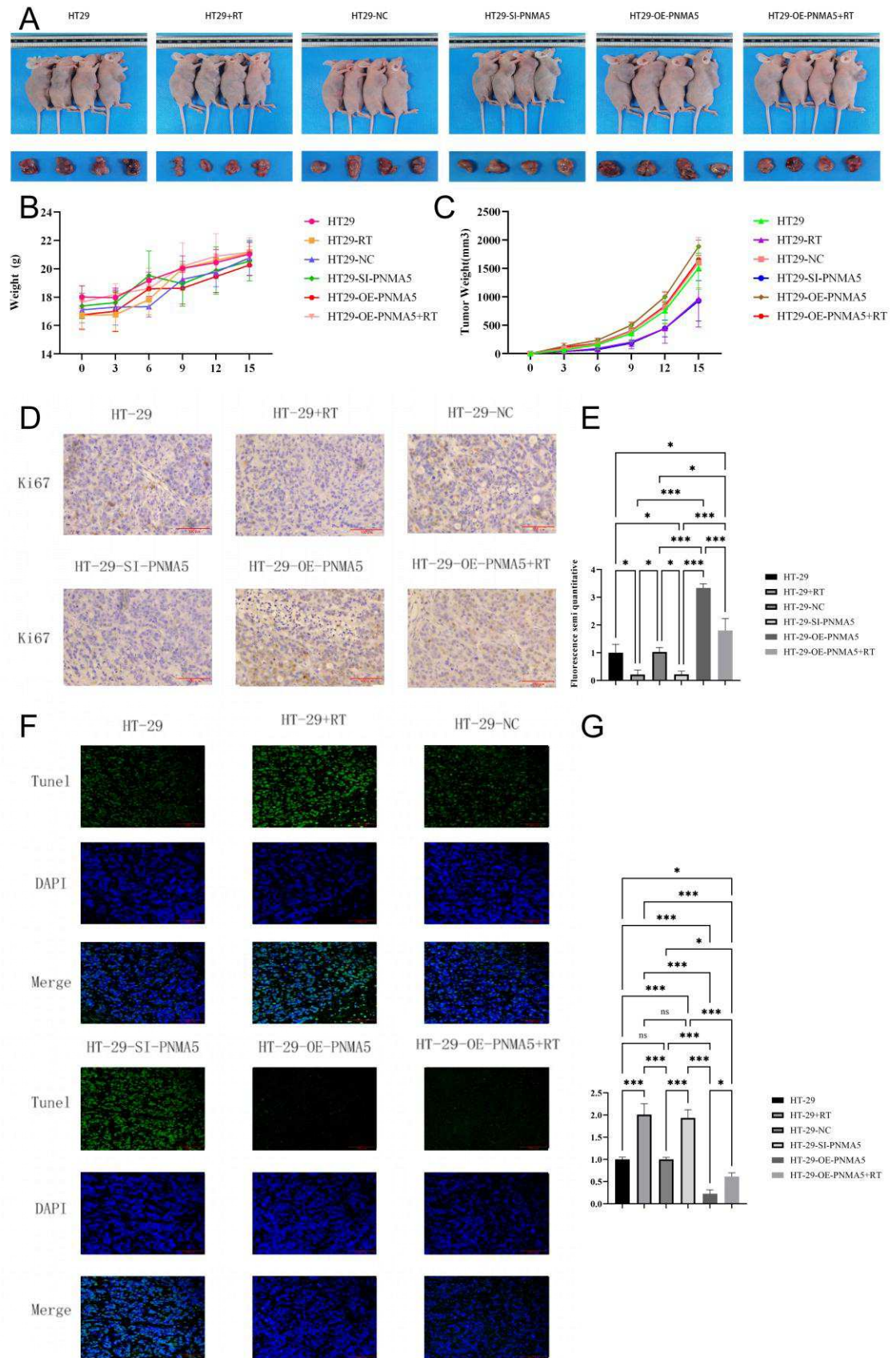


Figure 5. PNMA5 promotes tumor growth and induces radiotherapy resistance in CRC on living organisms. (A) Images depicting the final subcutaneous xenograft tumors taken from naked mice in

each group (n = 6 mice per group). The scale bar below indicates centimeters (cm). (B) Progressive tumor growth curves of each group monitored dynamically during the experimental period. The results show that PNMA5 overexpression significantly counteracts tumor growth inhibition induced by radiotherapy (RT). (C) Scatter-dot plot with bar chart showing the weights of percent-removed tumors from every group at the experimental endpoint, where each dot stands for the weight of a single mouse tumor. (D–E) Representative immunohistochemical (IHC) staining images and an analytic statistical study demonstrating the distribution and expression of Ki-67-positive cells (proliferation marker) in tumor tissues from each group. (F–G) Representative fluorescence visual representations and statistical evaluation of TUNEL staining in tumor tissues from each group to evaluate the apoptotic proportion of tumor cells under different circumstances of treatment. The values are displayed as mean $\pm$ SD, with ***P < 0.001 indicating statistical significance. (scale bar = 100 μ m)

Histological examination provided further insight. IHC staining showed Ki-67, a marker for cell proliferation, highest in the OE- PNMA5 group, and micro vessel density (MVD) marked by CD31 and CD34 was also way up—pointing to strong pro- proliferative and pro-angiogenic activities from PNMA5. At the same time, TUNEL staining confirmed that overexpressing PNMA5 significantly reduced radiation-induced cell death in the tumors (Fig. 5D–G). WB of tumor tissues further showed that VEGFA, VEGFR2, and other vascular markers were all elevated in the OEPNMA5 group, along with an imbalance in apoptosis regulators (Fig. 6A, B). Serum ELISA also revealed higher circulating levels of TGF- β 1 and FGF in the OE-PNMA5 group (Fig. 6C).

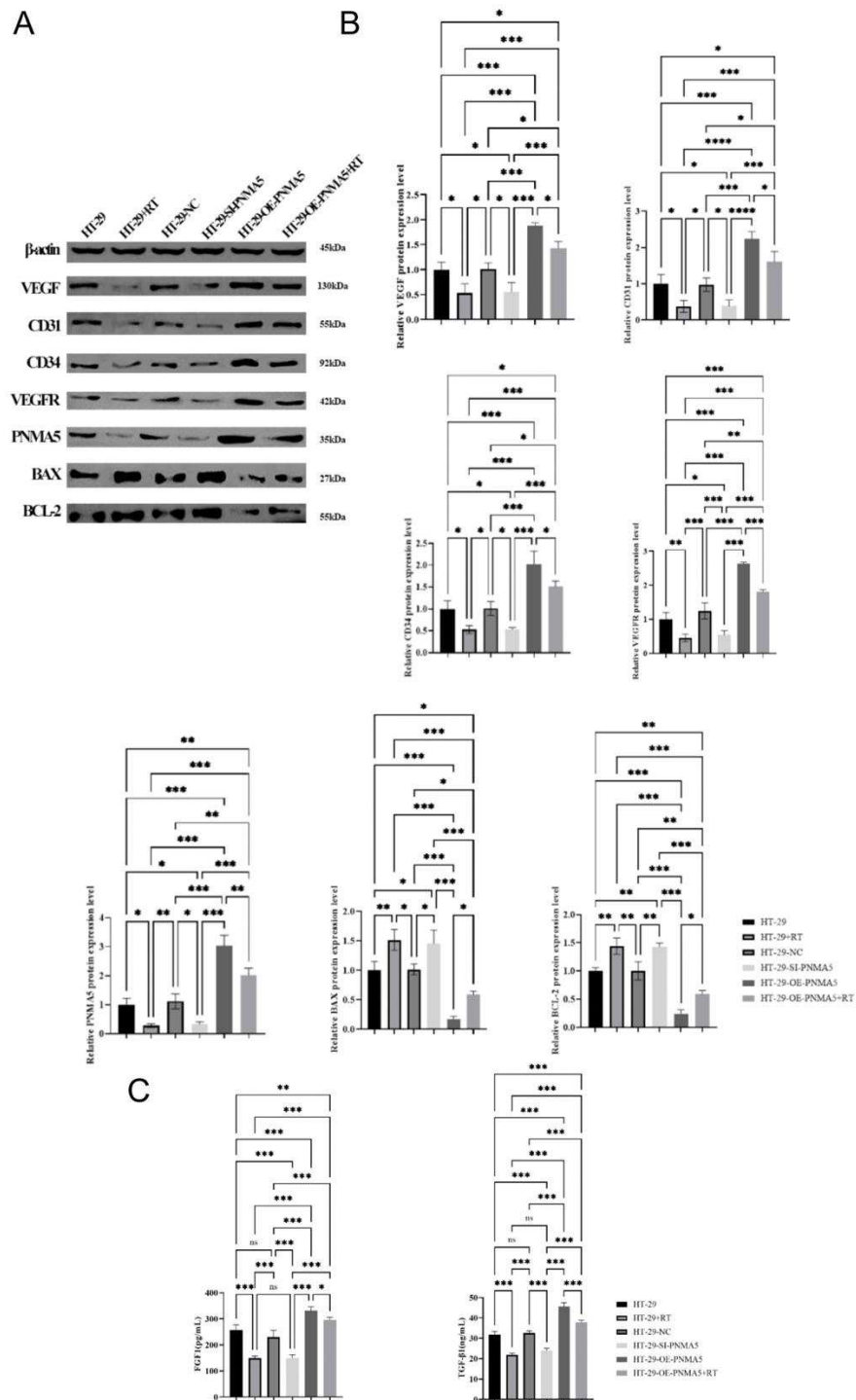


Figure 6. PNMA5 modulates the expression of proteins associated with apoptosis and angiogenesis and promotes the secretion of pro-angiogenic factors in vivo. (A) Representative Western blot bands of PNMA5, proteins associated with apoptosis (BAX and BCL2), and those related to angiogenesis (VEGFA and VEGFR2) in the harvested xenograft tumor tissues. (B) Quantitative densitometric analysis of the target proteins in tumor tissues: β -Actin serves as the internal loading control. (C) ELISA quantification of TGF- β 1 and FGF levels in the serum of nude mice from each group. Data are presented as mean $\pm$ standard deviation ($n = 6$ mice per group) and were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $P < 0.05$, $P < 0.01$,

****P < 0.001.** Cropped blots are displayed in the main figures. Full-length, uncropped blots with molecular weight markers and lane labels are provided in the Supplementary Information file.

In conclusion, the *in vivo* experiments strongly demonstrate that PNMA5 comprehensively mediates radio resistance in colorectal cancer by maintaining cell proliferative potential, suppressing apoptosis, and remodeling a pro-angiogenic microenvironment.

3.6 Transcriptomic Sequencing Reveals PNMA5-Mediated Immune Remodeling and Paracrine Regulatory Mechanisms

To explore the molecular basis by which PNMA5 induces radio resistance in colorectal cancer, whole-transcriptome sequencing was performed on the harvested xenograft tumor tissues. According to PCA, the samples taken from HT-29, HT-29-OE-PNMA5, and HT-29+RT groups clustered into three distinct categories with clear separation and high reproducibility, indicating excellent biological reliability of the sequencing data (Fig. 7A). Using thresholds of $P < 0.05$ and $|\log_2\text{FC}| \geq 1$, in comparison to the HT-29 group, 334 DEGs were found in the OE-PNMA5 group, with 245 genes turned up and 89 turned down (Fig. 7B). Hierarchical clustering heatmap analysis further confirmed the distinct expression profiles between the two groups (Fig. 7C). In the sequencing dataset, the mean FPKM value of PNMA5 mRNA increased from 0.0695 to 0.0772 upon overexpression, whereas radiotherapy made it less expressed (0.0456), a trend highly consistent with our *in vitro* findings.

We performed KEGG pathway analysis on the DEGs, and we found that the changed genes were mainly enriched in the "Immune system" and "Viral infection" categories, and their enrichment proportions were much higher than the background distribution (Fig. 7D); several important immune-related factors, including STAT1, IRF7, IFNB1, CXCL10, and CCL5, all showed significant increases in expression; Although the classic VEGF pathway was not statistically significant, the GSEA results further showed that the gene groups for "cellular response to interferon- β " (NES=3.23), "chemokine signaling pathway" (NES=2.04), and "apoptosis" (NES=2.03) were all significantly and positively enriched in the group with overexpression. (Fig. 7E).

Together with the higher secretion levels of VEGF and VEGF, FGF-1, and TGF- β 1 observed in our preceding experiments, these findings indicate that PNMA5 does not directly regulate VEGFA via transcriptional manipulation. Instead, as shown in the schematic (Fig 7F), PNMA5 turns on an immune/chemokine signaling network centered on STAT1/IRF7/CXCL10, which reshapes what the tumor cells secrete. These altered secreted factors then act on the surrounding microenvironment in a paracrine manner, indirectly boosting angiogenesis and blocking apoptosis. The result is radio resistance in CRC. This interaction between immunity and blood vessels, through paracrine means, indirectly promotes the formation of lumens by endothelial cells and the density of micro vessels in the body; this discovery has established a new atypical regulatory pattern between transcriptional data and data of proteins and secretions.

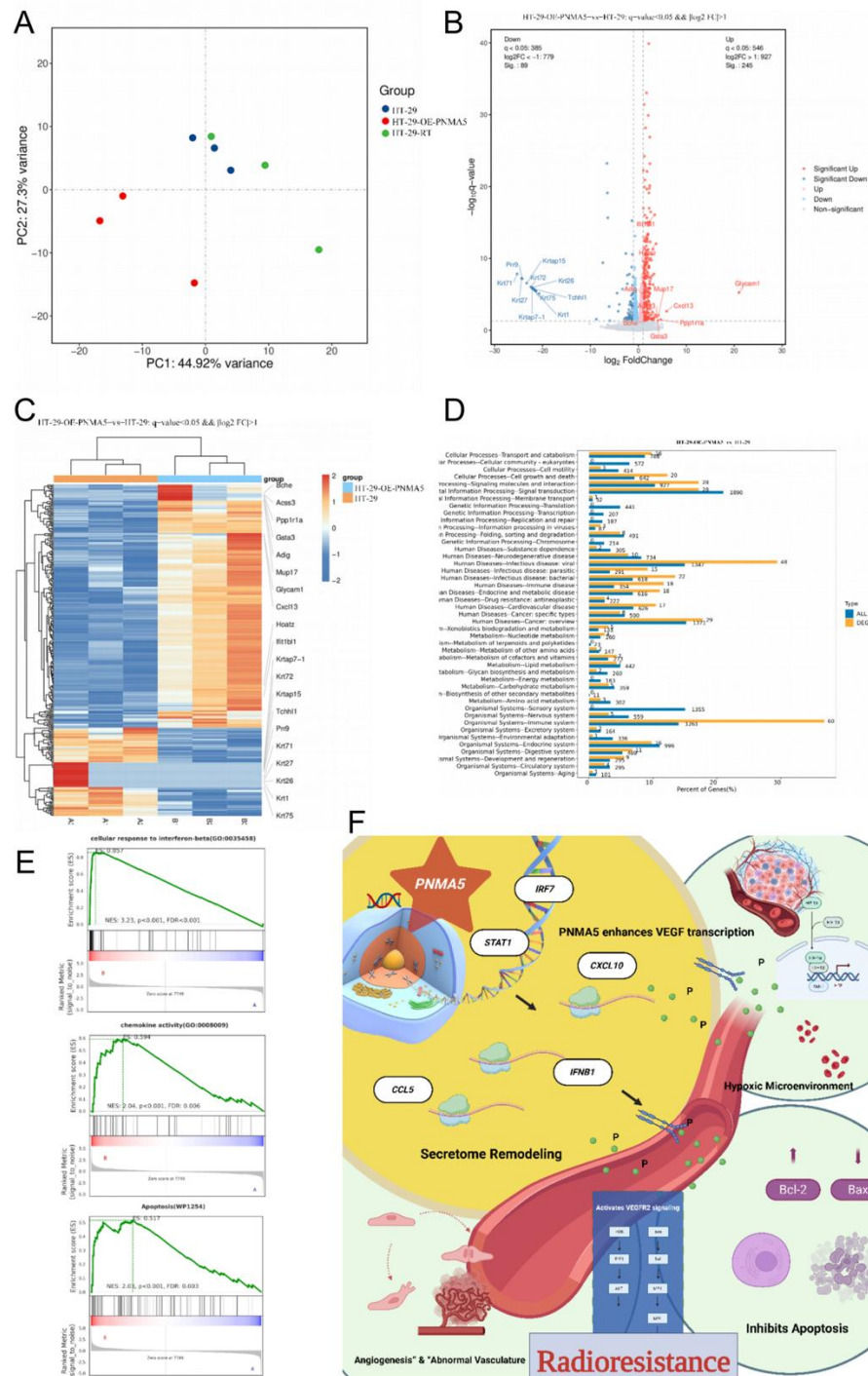


Figure 7. Transcriptomic profiling reveals PNMA5-driven immune remodeling and oncogenic pathways. (A) PCA plot of expression profiles from HT-29, OE-PNMA5, and RT groups, showing good repeatability within groups and clear distinction between groups. (B) Differentially expressed genes volcano plot between OE-PNMA5 and HT-29: 245 genes were upregulated, and 89 genes were downregulated. (C) Differentially expressed genes heatmap. (D) A KEGG enrichment study was conducted on the pathways related to immunity and viral infections. (E) The GSEA analysis revealed that in the overexpression group, the interferon- β response, chemokines, and apoptosis genes showed significant enrichment. (F) PNMA5 generates tumor factors through the STAT1/IRF7

pathway and then promotes angiogenesis and inhibits apoptosis in a paracrine manner, thereby causing radiotherapy resistance. The KEGG pathway map was reproduced or adapted with permission from Kanehisa Laboratories.

4. DISCUSSION

Radiation resistance limits the efficacy of CRC treatment. Its mechanism is not only related to the activation of anti-apoptotic signals within tumor cells but also influenced by changes in the external microenvironment. This study found that PNMA5 plays a key role in this process and explains that it activates the STAT1/IRF7 signal through a non-classical pathway, alters the composition of tumor secretions, and thereby indirectly causes angiogenesis. Therefore, PNMA5 may be a potential target for CRC's response to radiotherapy.

The function of PNMA in cancer is gradually being recognized. We found that PNMA5 can promote the proliferation of HT-29 cells and enhance their resistance to induced DNA damage and apoptosis. Mechanistically, PNMA5 upregulates BCL-2 expression and inhibits BAX, thereby leading to radio resistance. *In vivo*, PNMA5-overexpressing tumors-maintained growth after radiotherapy and retained high Ki-67 expression. Thus, PNMA5 acts as a pro-tumorigenic driver of cell-intrinsic radio resistance in CRC.

Beyond intrinsic tumor programs, the microenvironment acts as an extrinsic shield against radiation. PNMA5 overexpression drove secretion of VEGF, FGF-1, and TGF- β 1, and OE-PNMA5 conditioned medium enhanced endothelial tube formation. In xenografts, high PNMA5 expression correlated with increased CD31- and CD34-positive micro vessels. Thus, PNMA5 remodels a pro-angiogenic niche via paracrine signaling, which may undermine radiotherapy efficacy.

To clarify how PNMA5 affects angiogenesis without directly activating VEGFA transcription, a full transcriptome sequencing was conducted; the sequencing results and GSEA analysis revealed that the differentially expressed genes of PNMA5 were mainly concentrated in the interferon- β response and chemokine signaling pathways, with the expression of STAT1 and IRF7 being upregulated; although the classical interferon pathway is generally considered to have an anti-cancer effect, recent studies have pointed out that tumor cells, under the influence of radiotherapy or specific oncogenes, will continuously activate this pathway, thereby leading to interferon-related resistance characteristics; the continuous activation of the STAT1/IRF7 pathway becomes the central link of tumor secretome remodeling, as it does not directly initiate the transcription of the VEGFA gene, but changes the overall secretion of extracellular factors, thereby indirectly or synergistically promoting the release of factors such as VEGF, FGF-1 and TGF- β 1; based on this, we propose an atypical regulatory model, that is, after overexpression of PNMA5, it first activates the chronic interferon response network through an atypical pathway, and then induces paracrine pro-angiogenic factors by changing

the secretome; this unique immune-vascular interaction affects endothelial cells spatially and forms a decoupling and compensatory relationship between transcription and protein expression, and these factors together constitute the radiotherapy-resistant microenvironment of CRC.

In short, our work showed that PNMA5 contributes to CRC radiotherapy resistance in two aspects; the first aspect is that it regulates the internal balance of cell death. Secondly, it alters the composition of tumor secretions, thereby promoting angiogenesis through paracrine signaling. Therefore, PNMA5 has the potential to become a candidate molecule for reversing radiotherapy tolerance and conducting targeted intervention. However, regarding how it regulates the STAT1/IRF7 pathway at the post-transcriptional level, further experiments are needed to clarify this, such as using methods like co-immunoprecipitation. At the same time, in the future, it can be attempted to combine PNMA5-targeted therapy with radiotherapy for verification in vitro and in vivo, which may provide a new combined solution for addressing the problem of local radiotherapy resistance in clinical practice.

6. CONCLUSION

PNMA5 mediates radiotherapy resistance in colorectal cancer in two aspects. One is its synergistic effect with the intrinsic apoptosis inhibition of cells, and the other is its alteration of tumor secreted substances, which promotes angiogenesis through paracrine pathways; PNMA5 activates STAT1 and IRF7, thereby influencing the interconnection of the microenvironment and providing a new perspective for radiotherapy resistance.

7.Data Availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2025) in National Genomics Data Center (Nucleic Acids Res 2025), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA049043) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa>^[21-22].

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Conflicts of Interest: The authors declare that there is no conflict of interest.

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