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Inhibitor SYP-5 Suppresses Galectin-1 Expression Which Mediated by HIF-1 and its Effect in Mice Tumors

Ruoyi Song

School of Molecular Biosciences, Washington State University, Pullman, WA, 99163, USA.

Abstract

The hypoxia-inducible factor (HIF) -1 is a master factor for cellular response to hypoxia. It plays an essential role in carcinogenesis. Chalcone based inhibitor, like SYP-5, can inhibit the upregulation of HIF-1 protein to suppresses its expression. Moreover, a recent study pointed out the galectin-1, one of the most important lectins that participating in tumor development, is a direct target of the HIF-1. However, its exact mechanism and function in tumor growth still need further study. In this work, our evidence points out galectin-1 plays an essential role in tumor growth. In simulative mice tumors experiment, by controlling the variables and comparing the results, the experimental data proved that injection of chalcone-based compound SYP-5 could effectively inhibit the physiological activity of HIF-1 pathway, thus achieving the purpose of inhibiting tumor growth.

Keywords

HIF-1; Galectin-1; SYP-5; Inhibitor; Hypoxic Cancer.

1. Introduction

Pancreatic cancer is the most hypoxic cancer in all solid tumors[1]. The hypoxia inducted mechanism is one of the critical factors of malignant cell clones and develops, which derived by the anaerobic environment, mostly caused by reduced perfusion and anemia[2]. In this mechanism, the hypoxia-inducible factor (HIF)-1 has mediated the primary adaptive response of cells. This transcription factor first discovered in 1991[3] that it could be induced by hypoxia and activate specific DNA fragments. HIF-1 consists of two parts: HIF-1α and HIF-1β. Reduce oxygen content could stabilize HIF-1α and drive translocation into the nucleus, followed with heterodimer formation with its partner HIF-1β[2]. It was well known the gene of vascular endothelial growth factor (VEGF) is the target gene of HIF-1[4]. A recent study showed the galectin-1, one of the members of lectins is also a direct target of HIF-1[2]. The expression of HIF-1 can be inhibited by SYP-5. SYP-5 is a novel chalcone-based compound that inhibits HIF-1 by mediate the hypoxia-induced upregulation of HIF-1[4]. In this work, we selected the BxPC-3, Panc-1 and CF-PAC-1 pancreatic cancer cell lines, treat with SYP-5 to investigate the participation of galectin-1 in tumor development.

2. Methods

2.1 Materials and Reagent

The three cell lines BxPC-3, Panc-1, and CF-PAC-1 were obtained from the American Tissue Type Culture Collection. All other reagents, mice, antibodies, et al. were purchase from a certified company.

2.2 Cells preparation

The cells were grown in adapt circumstances. All cell lines were tested to be mycoplasma free. After 48 hours, 20ml cell culture fluid for each cell line was collected. Cell samples were spilted. Western blotting was done for cellular HIF-1, galectin-1 expression level confirmation.

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2.3 Mice tumor xenograft model and drug treatment

Fourteen-to-sixteen-week-old female mice with close weight were chosen in testing. Cancer cells in log cell growth were injured into mice. Every mouse except the control group was injured around 10^7 cells, which come from only one cell line. Totally twenty-four mice were chosen and grouped into four (group 1 for control, group 2 for BxPC-3, group three for Panc-1 and group four for CF-PAC-1) according to different cell lines. Mice in one group were feeding in the same cage with water and food of equal quantity and quality. The animals were weight weekly. Tumor size diameters were measured twice a week at right angles. When the average tumor volume (did not contain control volume) reached 100 mm^3, half mice in each group were injured normal saline with quantitative SYP-5 and VEGF, and remaining mice were injured normal saline with quantitative SYP-5. The dosage of SYP-5 and VEGF was calculated according to each mouse`s weight. After a mouth treatment, all mice were euthanized. Each tumor was collected, listed in order of size and group number.

2.4 Tumor volume measured and disposed

Tumor volume was measured and calculated as described previously. Each tumor was moved into an individual test tube and cut into pieces. Tumor pieces were treated with lysis buffer for digestion and protein extraction.

2.5 Western blotting assays

Loaded each cell sample in 10% sodium dodecyl sulfate-polyacrylamide gel for western blotting assays. Proteins were transferred to membranes after loading twenty minutes. Membranes were cut and immunoblotted with antibodies against HIF-1, E-cadherin (BD Transduction Laboratories), and galectin-1. E-cadherin was immunoblotted as the internal control, followed by the second antibody.

2.6 Statistical analysis

Statistical analysis was performed using Excel 2019. Unpaired t-tests were used when two groups of data were compared, Anova techniques technology was employed when multivariate analyses for groups of more than three were needed. Data were presented as the mean +/- s.e.m. with a probability level of P 0.05 considered statistically significant. All experiments were repeated at least three times with similar results.

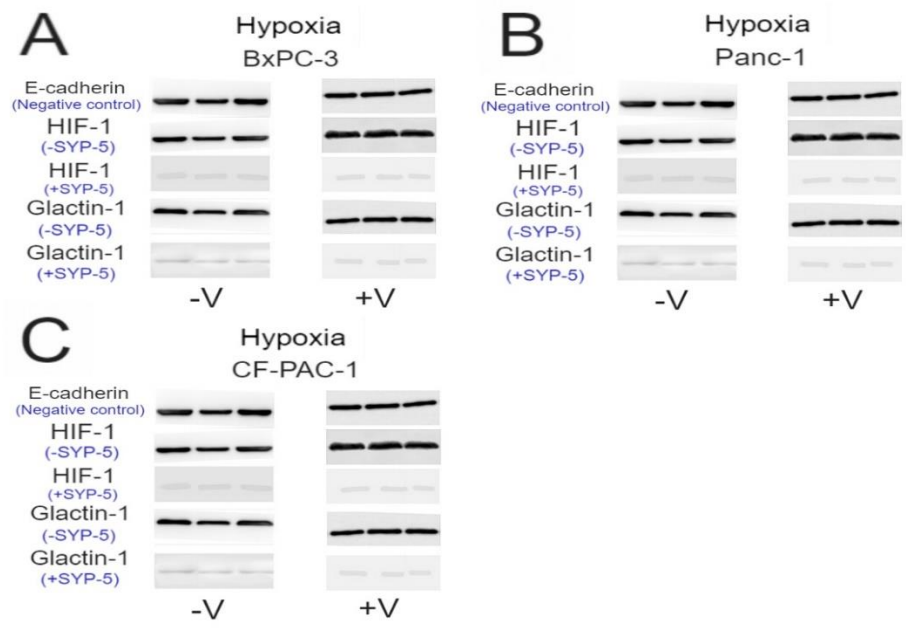

Fig.1 In the western blotting assay, E-cadherin was immunoblotted as the internal control. (A) was the result of cell line BxPC-3, (B) was the result of cell line Panc-1, and (C) was the result of cell line CF-PAC-1. Letter V refers to VEGR. Immunoblotted analysis pointed out the expression level of HIF-1 and galectin-1 in SYP-5 treated cells was visibly lower.

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3. Result

3.1 SYP-5 treated tumor presented lower HIF-1 and galectin-1 expression levels.

As a previous study reviewed, the higher expression level of HIF-1 is an essential indicator in clinical oncology[1]. Galectin-1 also showed higher expression level in solid tumor and the HIF-1 driven galectin-1 mechanism also has been demonstrated in gene and protein level[2]. In fig.1, the results showed in SYP-5 treated tumor cells, the expression level of both HIF-1 and galectin-1 was lower.

3.2 VEGF participated in tumor growth

VEGF is the key factor in angiogenesis. Previous studies supported HIF-1 could regulate the expression of this molecule, and the upregulated of VEGF showed a positive effect in cancer development[4]. In mice tumor xenograft model, one mouse in each group was given extra VEGF. The result supported VEGF provided an extra assist in mice tumors (fig.2).

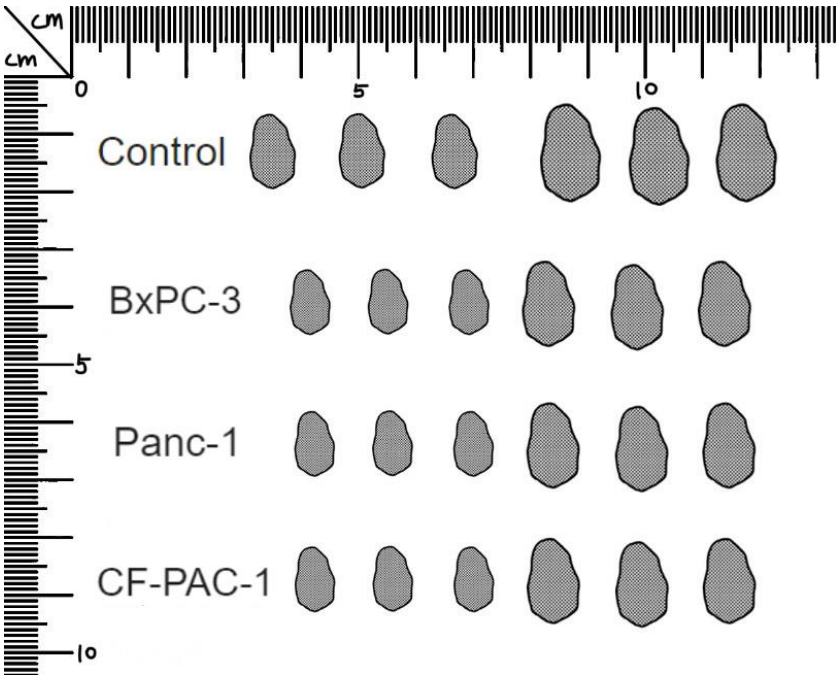

Fig.2 Tumor sample grew in mice. From top-down, control group, BxPC-3 cell, Panc-1 cell, CF-PAC-1 cell. The leftmost tumor in each line was given extra VEGF.

3.3 Down-regulated expression of galectin-1 inhibits tumor growth

In mice tumor xenograft model, SYP-5 inhibited the expression of HIF-1 proteins, and down-regulated expression level of galectin-1. The lack of galectin-1 proteins markedly impaired tumor growth (fig.2).

4. Discussion

The HIF-1 is a hypoxic regulated regulatory factor. It bounds with a specific gene segment to upregulate its expression. Many evidences supported that galectin-1 and VEGF are both target genes for HIF-1[2,4]. In the mice tumor xenograft model, mice were given SYP-5. SYP-5 is an inhibitor that down-regulated HIF-1 protein level[4]. Weekly injected SYP-5 suppresses galectin-1 expression by mediated HIF-1. The lack of galectin-1 showed specific harm on the tumor, average volume of tumors decreased over 40%. Moreover, VEGF is also suppressed by SYP-5. To study the function of galectin-1 in tumor growth, one mouse was given extra VEGF. Evidence showed tumors with additional VEGF got less damaged. This could support galectin-1 plays a similar role with VEGF, which participated in angiogenesis, but this remains to be further investigated.

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5. Conclusion

The inhibitory effect of SYP-5 on HIF-1 pathway was inferred through the simulation experiment of control variables. Due to the important role of HIF-1 pathway in the development of hypoxic tumor, the development of inhibitors is of great significance for the study of treatment. The HIF-1 pathway is currently being studied. However, further studies needed to understand its mechanisms. In this simulation experiment, it is inferred that SYP-5 may have inhibitory effect, which is of positive significance for clinical experimental research and lays a foundation for future development.

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