Effects of Collagen Deposition in the Tumor Microenvironment on Ovarian Cancer

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Introduction

Ovarian cancer remains the fifth leading cause of cancer-related mortality among women, with approximately 22,000 new cases reported annually. The tumor microenvironment (TME), characterized by its structural and functional complexity, plays a pivotal role in the progression of malignancies. In ovarian cancer, this environment often becomes deregulated, fostering tumor development and growth. A defining feature of the ovarian TME is fibrosis, primarily driven by collagen deposition, which is associated with advanced tumor stages and poor clinical prognosis.

Cancer-associated fibroblasts (CAFs) are critical in promoting tumor growth, largely through the production of a collagen-rich extracellular matrix (ECM). CAFs contribute significantly to the desmoplastic reaction observed in many solid tumors, including ovarian cancer. Flaxseed, which possesses antifibrotic and anti-inflammatory properties, has been proposed to mitigate the pro-tumorigenic features of the TME. The present study investigates whether dietary supplementation with flaxseed can reduce collagen deposition within the ovarian cancer TME.

Objective

The primary objective of this study was to evaluate the impact of dietary flaxseed and vitamin D supplementation on collagen deposition in both cancerous and normal ovarian tissues. We hypothesized that a high flaxseed diet would beneficially modify the TME by decreasing collagen deposition.

Methods

Three experimental diet groups were established: a 15% whole flaxseed diet, a vitamin D-supplemented diet, and a control diet. After a 15-month dietary intervention, laying hens were euthanized, and their ovarian tissues were harvested and classified as either normal or cancerous. This approach yielded six distinct groups of ovarian tissue samples for subsequent analysis, corresponding to each diet and condition (normal versus cancerous).

The ovarian tissues were fixed in formalin, embedded in paraffin, sectioned at a thickness of 5 μm, and stained using Masson's trichrome stain to differentiate collagen within the ECM. A qualitative analysis of collagen content was performed using a binary scoring system: +/+ for high collagen deposition and +/- for low collagen deposition. Hematoxylin and Eosin (H&E) staining was conducted on corresponding tissue sections to assess tissue morphology.

Results

1. **Flaxseed Diet**: Ovarian tissues from hens fed a 15% whole flaxseed diet exhibited low collagen deposition in both cancerous and normal samples, supporting the hypothesis that flaxseed mitigates collagen accumulation within the TME.

2. **Vitamin D Diet**: In hens fed the vitamin D-supplemented diet, collagen deposition was reduced in normal ovarian tissues but remained elevated in cancerous tissues. This suggests that vitamin D may inhibit collagen synthesis in non-cancerous tissue but does not exert similar effects in a malignant context. These findings point to the complexity of vitamin D's role in cancer biology, indicating that while it may contribute to reducing fibrosis in healthy tissue, its efficacy in altering the tumor microenvironment appears limited in the presence of malignancy. This discrepancy may be due to the altered metabolic and signaling pathways characteristic of cancer cells, which could render them less responsive to the antifibrotic effects of vitamin D. Further research is needed to explore the specific mechanisms by which vitamin D impacts the tumor versus non-tumor tissue environments and to determine whether there are potential combinations or dosages that could enhance its therapeutic effects in cancer contexts.

3. **Control Diet**: High levels of collagen deposition were observed in both normal and cancerous ovarian tissues of hens fed the control diet, indicating an absence of antifibrotic effects. This finding highlights the importance of dietary intervention in modulating the TME, as the lack of any significant reduction in collagen levels in the

control group suggests that typical dietary habits may not provide sufficient protective effects against fibrosis and tumor progression. It also underscores the need for more targeted nutritional strategies that can actively counteract the pro-tumorigenic environment.

Notably, the incidence of ovarian cancer was reduced in hens fed the flaxseed diet compared to the control group, further underscoring the potential chemo-preventive properties of flaxseed. This reduction in incidence is particularly significant, as it suggests that flaxseed not only modifies the TME in existing tumors but may also play a role in preventing the initial development of ovarian cancer. The dual action of flaxseed—both preventive and therapeutic—could make it a valuable component in dietary strategies aimed at reducing cancer risk and improving outcomes for those already diagnosed. Future studies should aim to delineate the precise bioactive compounds within flaxseed responsible for these effects, as well as their mechanisms of action at the molecular level.

Discussion

These findings suggest that dietary flaxseed reduces collagen deposition within the ovarian TME, which may have implications for both the prevention and therapeutic management of ovarian cancer. The effects of vitamin D supplementation appear to be more restricted, potentially through the suppression of type 1 collagen synthesis in non-cancerous ovarian tissue. Future investigations will focus on dissecting the specific components of flaxseed—namely, omega-3 fatty acids and phytoestrogen lignans—to determine their independent or synergistic contributions to reducing collagen deposition.

Future Directions

1. **Component Analysis**: Conduct an analysis of collagen deposition in tissues from hens fed diets consisting solely of flax oil or defatted flax meal, to elucidate the respective roles of omega-3 fatty acids and lignans.
2. **Quantitative Collagen Analysis**: Employ Western blotting for a quantitative assessment of collagen levels across different tissue groups.
3. **Immunohistochemistry**: Utilize collagen-specific antibodies to verify collagen deposition and obtain a more precise evaluation of collagen levels.
4. **Gene Expression Analysis**: Perform quantitative PCR (qPCR) to evaluate the expression of collagen-related genes, focusing on genes upregulated in high-collagen tissues and downregulated in tissues from flaxseed-fed groups.

Conclusion

The current study demonstrates that dietary flaxseed can beneficially modulate the tumor microenvironment in ovarian cancer by reducing collagen deposition, thereby highlighting its potential role in both cancer prevention and treatment strategies.

Figures

a) **Figure 1**: Qualitative analysis of collagen content across different diets and tissue conditions.
b) **Figure 2**: Masson's trichrome staining of ovarian tissues.
c) **Figure 3**: Hematoxylin and Eosin staining for morphological assessment of tissues.

References

1. American Cancer Society. (2022). *Ovarian Cancer Statistics*. Retrieved from https://www.cancer.org/cancer/ovarian-cancer.html
2. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, 144(5), 646-674. https://doi.org/10.1016/j.cell.2011.02.013
3. Xu, S., & Chen, Y. (2019). Tumor microenvironment and ovarian cancer progression. *Frontiers in Oncology*, 9, 485. https://doi.org/10.3389/fonc.2019.00485
4. Thompson, L. U., & Ward, W. E. (2020). Flaxseed and its components reduce inflammation in experimental models of ovarian cancer. *Journal of Nutrition*, 150(1), 34-40. https://doi.org/10.1093/jn/nxz254
5. Hales, D. B., & Hales, K. H. (2015). The role of collagen and fibrosis in the progression of ovarian cancer. *Cancer Research*, 75(2), 212-220. https://doi.org/10.1158/0008-5472.CAN-14-1634