Dietary effects on collagen deposition in the tumor microenvironment of ovarian cancer

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Introduction

Ovarian cancer is the 5th most deadly cancer among women, with approximately 22,000 new diagnoses per year. The interaction between cancer cells and the tumor microenvironment (TME) impacts the progression of tumors greatly. The TME is a complex heterogeneous system, that functionally and structurally supports adjacent cells. In ovarian cancer, the TME can be highly deregulated and have pro-cancer properties. Inflammation within the ovary is also known to be a driver of ovarian cancer formation and progression. This is in part due to incessant ovulation driving an increase in the reparative fibrotic process. An ovarian tumor with high levels of fibrosis, primarily due to collagen up regulation, have been correlated with later stages of ovarian cancer and a poor prognosis. A central component of the TME are fibroblast cells which are a major source of acellular tissues like collagen, that comprise the extracellular matrix (ECM). In cancerous tissues, normal fibroblasts will develop into cancer-associated fibroblasts (CAFs) as the cancer progresses. Highly active CAFs produce a collagen-rich ECM that increases tumor stiffness. An increasingly collagen-rich ECM is characteristic of desmoplastic tumor growth. Flaxseed is known to have properties that decrease the level of fibrosis, a collagen mediated process, in ovarian cancer; as well as anti-inflammatory properties. Both properties aid in making the microenvironment less hospitable to cancer cells. Raising the question, does a high flaxseed diet decrease tumor fibrosis, and if so how. The laying hen animal model was used to investigate this question as they develop ovarian cancer spontaneously, just as women do, and thus are uniquely suited for dietary modulation research. Collagen was chosen as the target of our histological approach because of its central role in fibrosis formation, desmoplastic tumor growth, and because its production level is linked to the activity level of CAFs. This study's objective was to test dietary effects, on collagen deposition levels in the microenvironment of cancerous and normal ovaries. We have hypothesized that dietary modulation with a high flaxseed diet beneficially alters the TME by affecting collagen deposition levels.

Methods

Three different diet groups were used to test for dietary modulation effects in ovarian cancer. These consisted of a 15% whole flaxseed diet, a vitamin D supplemented diet, and a control diet. Following 15 months of dietary intervention, the hens were sacrificed and necropsied to extract ovary tissues, and several other tissue types for study. During necropsy, the hen's ovaries were extracted and determined to be normal or cancerous. This determination of the ovaries, from all three diets, resulted in six groups of ovary tissue samples for study. After tissue harvesting, we used a histological approach to assess dietary effects on collagen deposition. For this approach, tissue fixation was the first step in processing the collected ovaries. After fixation, the tissue samples were embedded in paraffin, and 5µm sections were stained with trichrome. Masson's trichrome staining was performed on all six groups of tissue sections to enable collagen visualization. Masson's trichrome is a three color histology stain, making the connective tissue readily distinguishable from surrounding cells. The three color stain was achieved using Weigert's Iron Hematoxylin working solution, to stain cell nuclei dark purple. Biebrich Scarlet-Acid Fuchsin solution, to stain cell cytoplasm, and erythrocytes red. The third and final color stain was achieved using an Aniline blue solution, which stains collagen blue. To assess the results, a qualitative analysis of collagen content was performed by marking samples with high levels of collagen as +/+; and marking samples with low levels of collagen deposition as +/-. A Hematoxylin and Eosin (H&E) stain of corresponding tissue samples, that had been stained with trichrome, was performed to show the morphology of the ovarian tissues in normal and cancerous ovaries.

Results

Assessment of current samples from the six groups led to a positive correlation between collagen deposition and cancerous ovaries, except in cancerous ovaries from hens that were fed a high flaxseed diet. Which showed low levels of collagen deposition, in all samples assessed thus far. In addition, all normal ovary tissues assessed so far were determined to contain low levels of collagen, except in the hens fed a control diet. Which showed high levels of collagen deposition even when the ovary was noncancerous.

Disease	Diet	+/+	+/-
Cancer	Control	1	0
	Whole Flax	0	2
	Vitamin D	1	0
Normal	Control	1	0
	Whole Flax	0	2
	Vitamin D	0	1

Figure 1: Qualitative analysis of collagen content from current samples via +/+ and +/- scale of all diet groups, from normal and cancerous ovary tissues.

Discussion

Qualitative assessment of collagen in the ovaries of hens that were fed a 15% whole flaxseed diet revealed low levels of collagen deposition in both cancerous and normal ovaries. Supporting our hypothesis that a high flaxseed diet affects collagen deposition in the microenvironment. The assessments also revealed that the control diet had no effect on collagen deposition levels, which was suggested by the high levels of collagen deposition seen in the tissues of both cancerous and normal ovaries. The assessment of collagen content in ovaries of hens fed a vitamin D supplemented diet revealed low levels of collagen deposition in normal ovaries of hens on this diet. These findings could be due to vitamin D's suppressive effect on type 1 collagen synthesis. However, it also revealed high levels of collagen deposition in cancerous ovaries of hens on this diet. Further study is needed to investigate if vitamin D serves a preventative role in the development of cancer by affecting processes involved in up regulating collagen deposition. In addition, the vitamin D diet did not decrease the incidence rate of ovarian cancer in hens on this diet compared to the control diet. However, the hens on the flaxseed diet did have a decreased rate of ovarian cancer incidence, when compared to the control diet. Further supporting the preventative effects of flaxseed on ovarian cancer development. Flaxseed is known to have properties that decrease inflammation and the level of fibrosis, a collagen mediated process in ovarian cancer. Both properties confer effects that make the TME less hospitable to cancer cells. Suggesting that modulation of the TME, via flaxseed, may serve a critical role in the prevention as well as the treatment of ovarian cancer. Future work will involve further study of collagen deposition in ovary tissues, using trichrome, from more tissue samples across all six groups. The two primary components of flaxseed will be investigated in future experiments by conducting collagen deposition analysis in tissues from hens that were fed a strict flax oil diet (high in omega-3 fatty acids), or a defatted flax meal diet (high in phytoestrogen lignans). To elucidate whether the omega-3 fatty acid component or the phytoestrogen lignin component, of flaxseed, is mediating the inhibitory effects on collagen deposition or whether the two components work synergistically to convey these effects on collagen deposition. Another future aspect of this study will be to conduct quantitative examination of collagen deposition levels via Western Blot analysis, to obtain a more in depth assessment of collagen levels in the various tissue groups. Verification of collagen deposition using immunohistochemistry is another future aim, to provide a more precise assessment of collagen levels using collagen specific antibodies. Another future experiment, following quantitative assessment of collagen levels, is to examine collagen gene expression levels using qPCR techniques. In order to assess which genes are up regulated in tissue samples containing high levels of collagen deposition. As well as to investigate whether there are down regulated genes in the tissues containing low levels of collagen. Particularly in the tissues from hens fed a flaxseed diet, as the collagen expression was low in these tissues regardless of whether the ovary was cancerous and normal.

Masson's Trichrome Stain

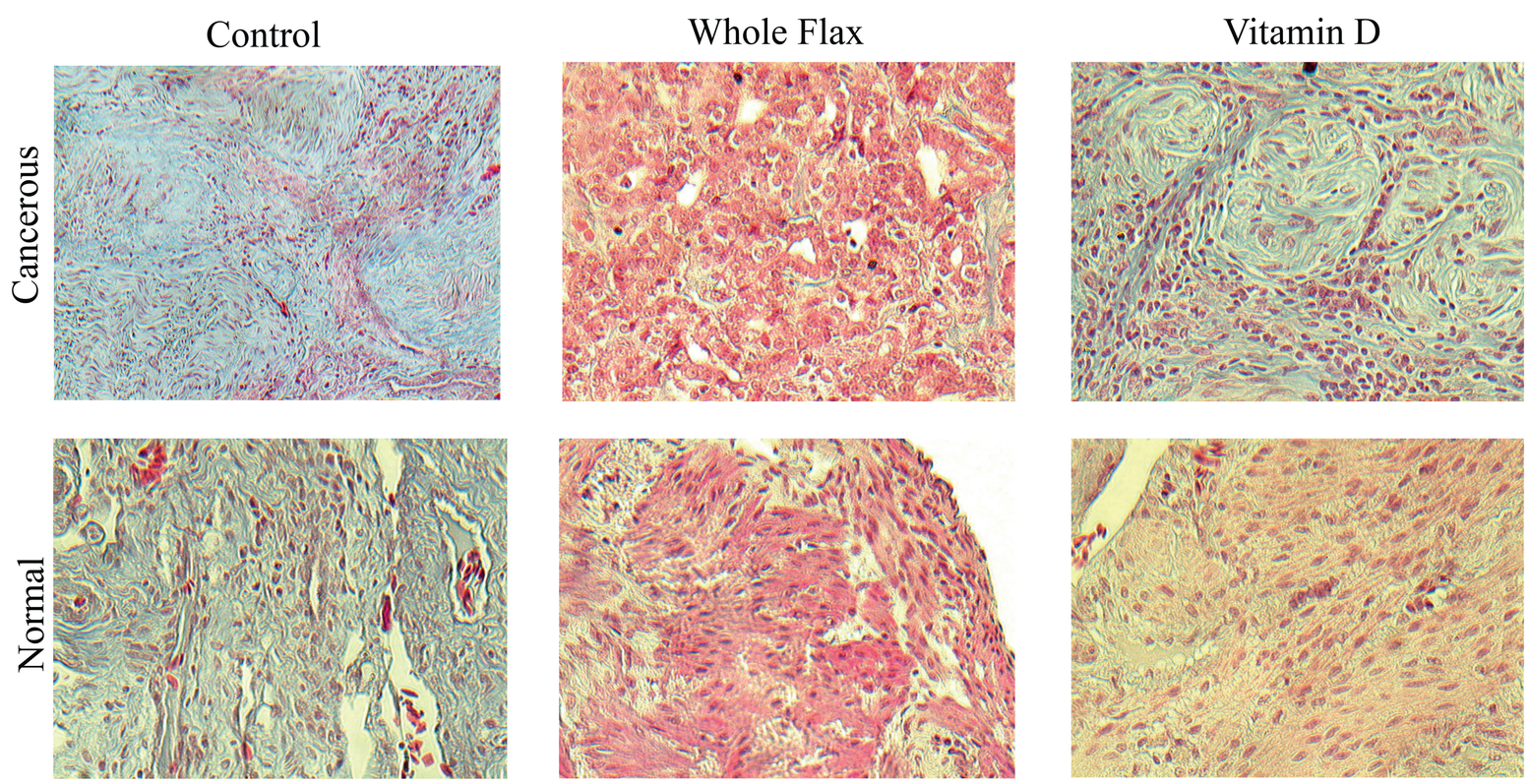

Figure 2: Masson's trichrome staining of ovary tissues, that were cancerous or normal from the three different diet groups. Collagen levels were assessed to be high in the normal and cancerous ovaries from the control diet. The collagen levels were found to be low in the cancerous and normal ovaries of hens that were fed a 15% whole flaxseed diet. Collagen levels were found to be high in the cancerous ovary of hens fed the vitamin D supplemented diet. However, collagen assessment was found to be low in the normal ovaries from hens that were fed a vitamin D supplemented diet.

H&E Stain

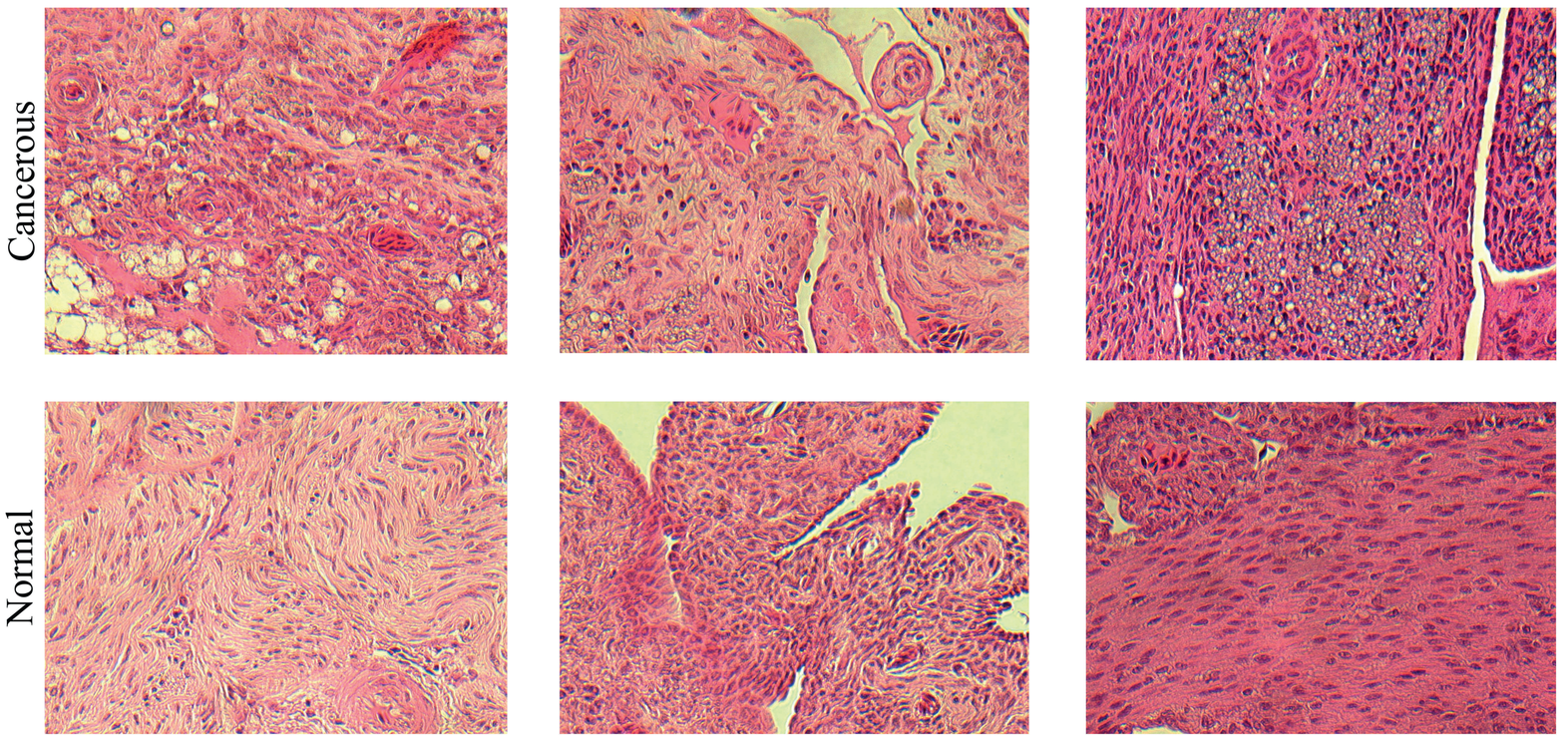

Figure 3: Hematoxylin and Eosin (H&E) staining of corresponding ovary tissue samples that were stained with trichrome (Fig. 2). The corresponding tissue slides were H&E stained in order to provide a view of the morphological characteristics of the trichrome stained tissues that were normal or cancerous.