



## Introduction

Ovarian cancer is the 5th most lethal cancer in women. The periodic ovulation cycle triggers wound healing process resulting in chronic inflammation and fibrosis. Fibrosis is associated with ovarian cancer. The endocannabinoid system (ECS) is involved in inflammation and fibrosis and is associated with different malignancies. CB1 is involved in inflammatory and fibrotic responses, while CB2 activation results in an anti-inflammatory and anti-fibrotic environment. The role of the ECS in ovarian cancer is unclear. Hens spontaneously develop ovarian cancer, hence are an excellent natural model. We have shown that a flaxseed supplemented diet delayed the onset and severity of ovarian cancer in laying hens: reduces the tumor angiogenesis and modifies the metabolism of estrogen. Therefore, we hypothesize flaxseed reduces fibrosis. The objective of this study is to determine the relationship between fibrosis and the ECS in ovarian cancer and the anti-fibrotic actions of flaxseed.

## Methods

Normal chicken ovaries and ovarian tumors from control and flaxseed supplemented diet were immunohistochemically stained for CB1 and E-cadherin. Picro Sirius Red staining was done to assess the presence of fibrosis and collagen amount and arrangement. Hydroxyproline assay determined collagen concentration. PCR array to measure COL1A1 and COL1A2 mRNA.

### CB1 is upregulated in ovarian cancer

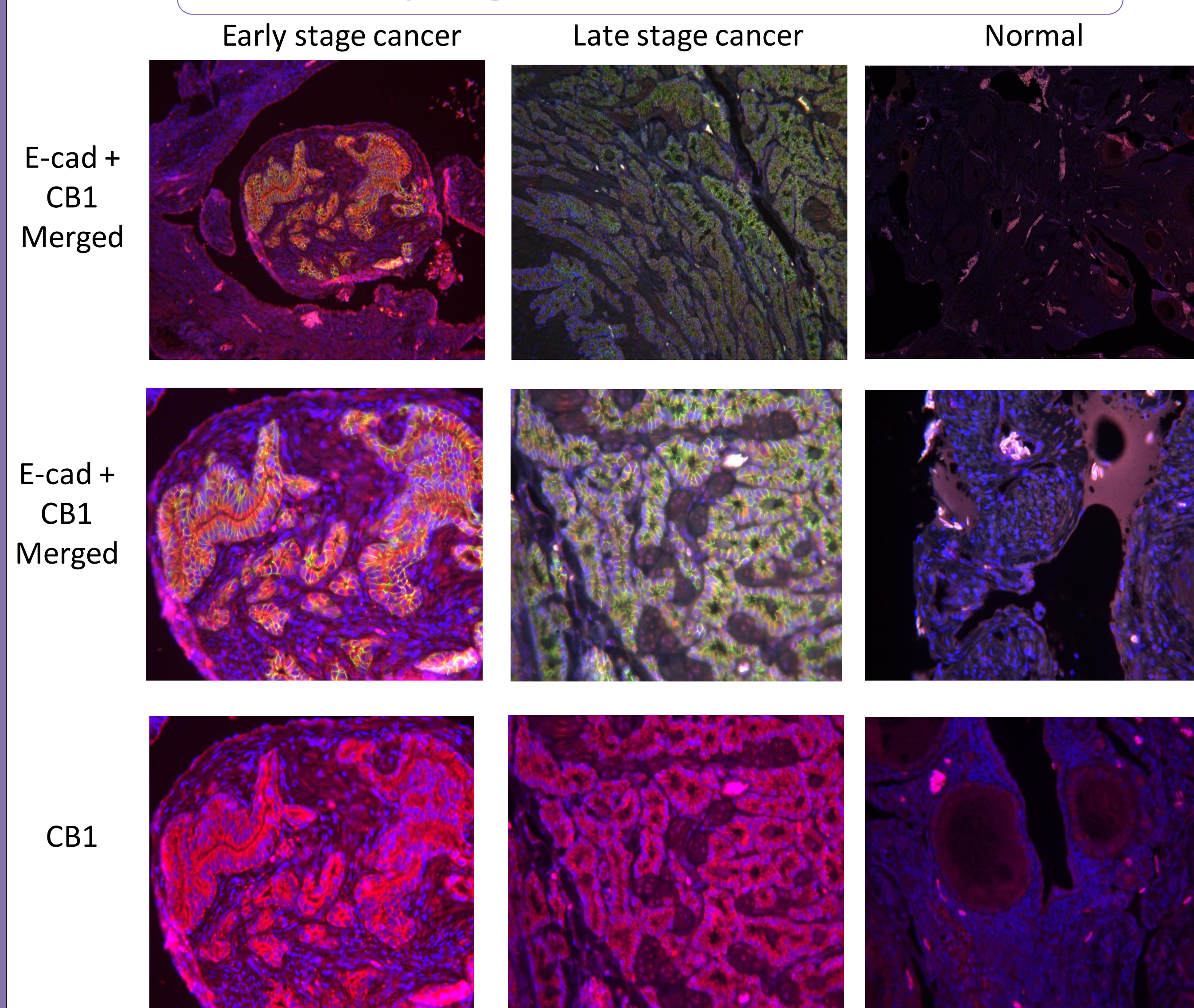


Figure 1: Immunohistochemistry on chicken ovaries and ovarian tumors against CB1 antibody (Red) to investigate its presence in ovarian cancer. E-cadherin immunostaining (Green) was concomitantly performed to discern the tumor niche. CB1 positive staining was enhanced in early and late stage cancers.

## Results

Our study shows that the presence, distribution, and arrangement of collagen were modified in ovarian cancer, presenting fibrosis. Cancer tissue expressed significant fibrosis intratumorally and at tumor surroundings. While both expressed higher collagen concentration than normal tissues, collagen linearization was associated with a control diet. A flax-fed diet was correlated with improved collagen deposition and collagen isotropic organization. CB1 receptors were upregulated in the tumor area in early and late stages ovarian cancer. Fibrosis and CB1 upregulation were correlated. Collagen concentration was increased in cancer in the control diet and markedly decreased in the flaxseed diet.

### Flaxseed diet reduces fibrosis in ovarian cancer

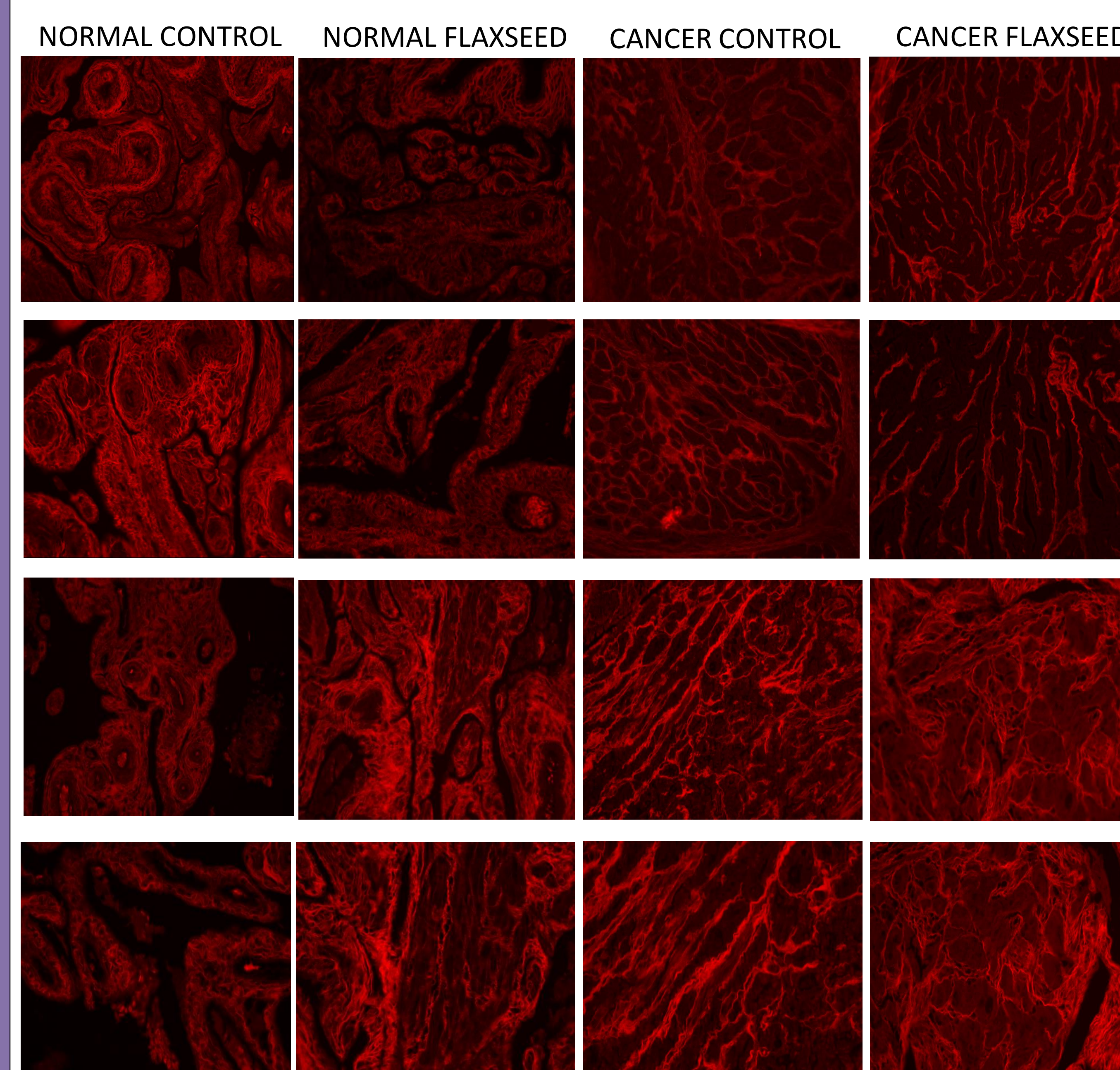


Figure 2 (A): Picro Sirius Red staining on normal ovaries and ovarian tumors from control and whole flax diet to identify deposition and structure of collagen fibers. Whole flax diet was associated with reduced presence of collagen fibers and collagen isotropic structure, opposed to collagen linearization found in control diet ovarian cancer.

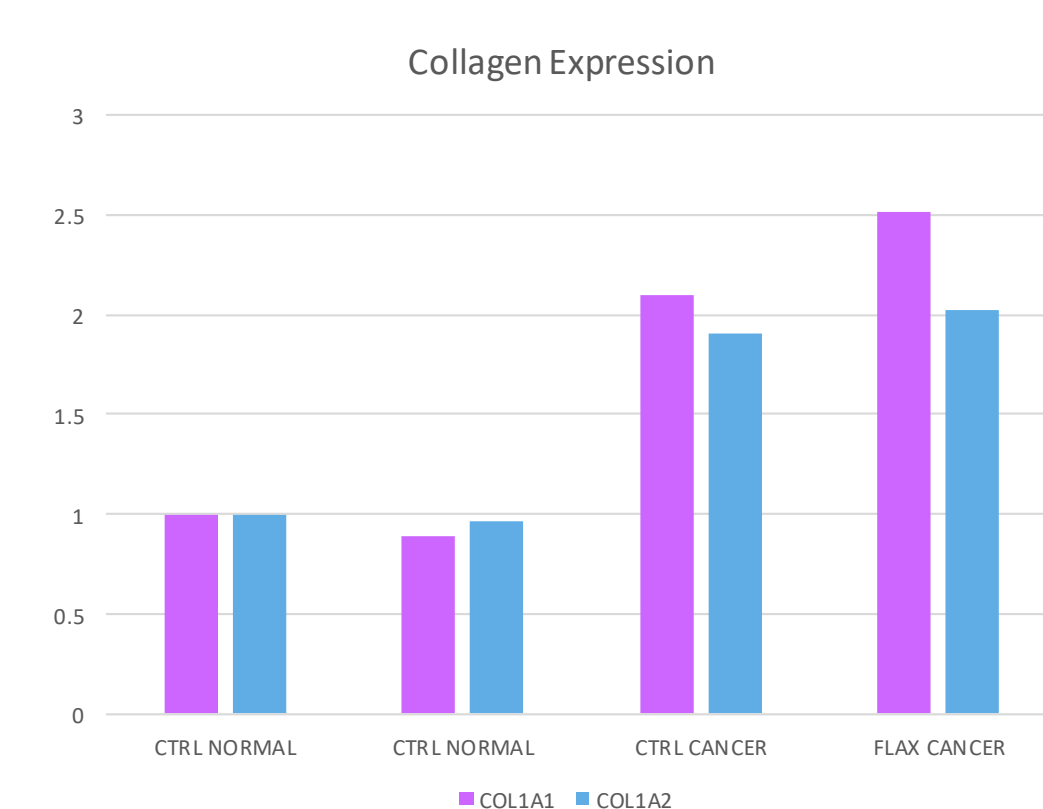
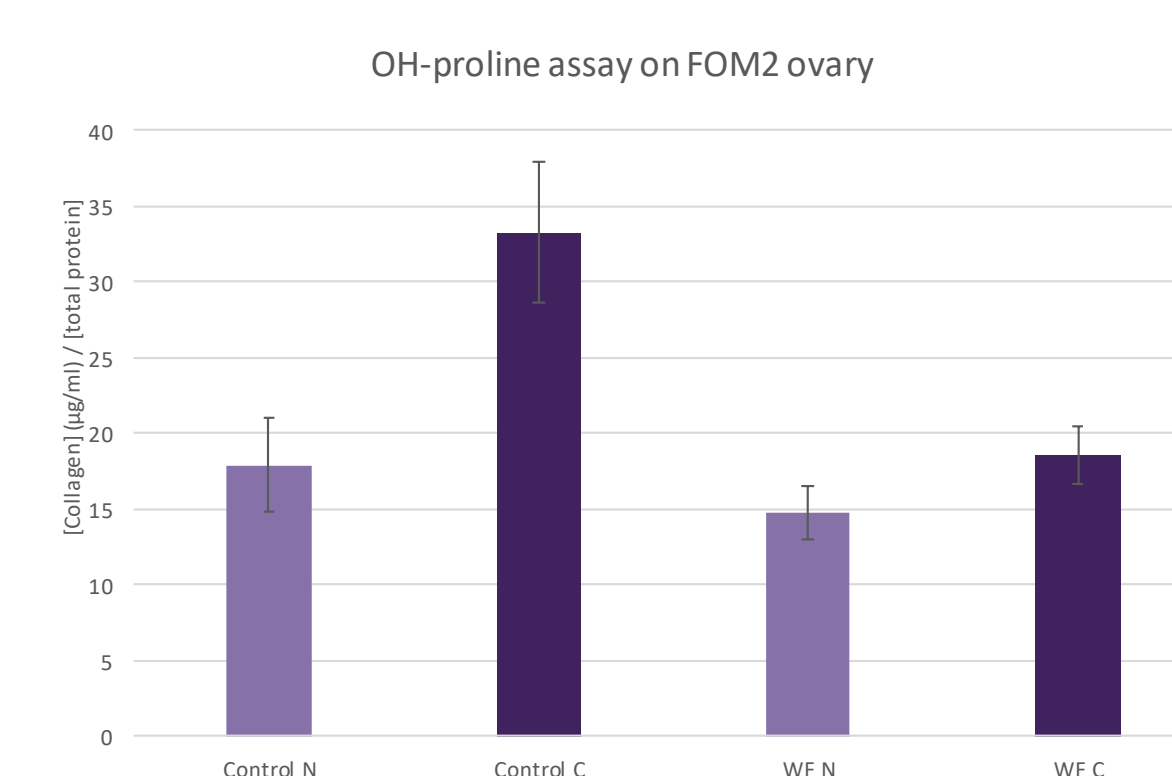


Figure 2 (B): Collagen expression measured by PCR showed presence of COL1A1 and COL1A2 increased in ovarian in both diets.

Figure 2 (C): Collagen concentration measured by hydroxyl-proline assay in ovarian tumors was significantly downregulated in whole flax diet. Two way ANOVA, error bars: SEM,  $p < 0.05$ .



### Spatial distribution of collagen, E-cadherin and CB1 receptor

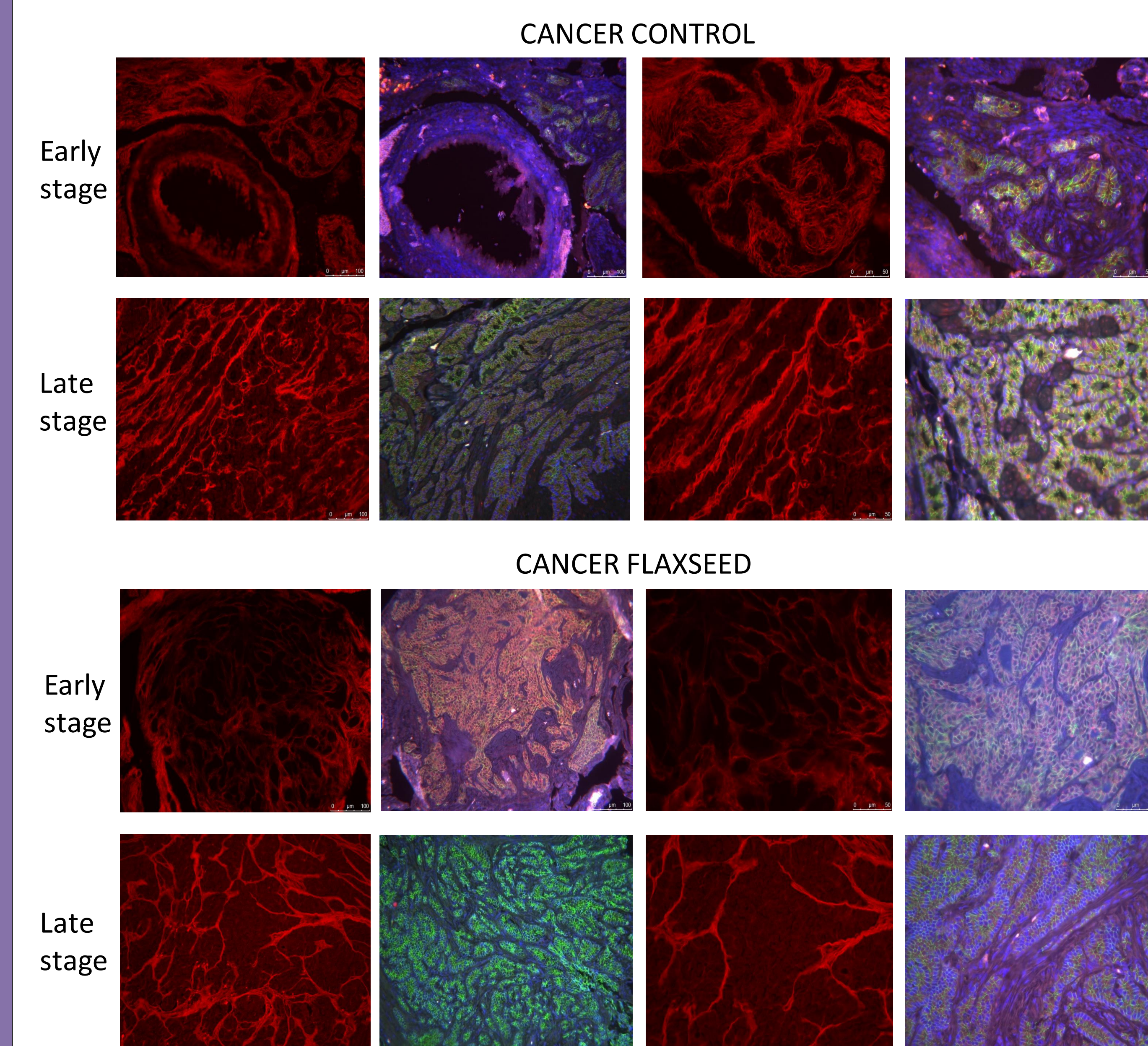


Figure 3: Immunohistochemistry on hen ovarian tumors against an anti-chicken CB1 antibody (Red) to investigate its presence and distribution in ovarian cancer. E-cadherin immunostaining (Green) was simultaneously performed to delimit cancerous region. Picro Sirius Red staining on ovarian cancer from control and whole flax diet evaluate collagen fiber properties. CB1 positive staining was enhanced in early and late stage ovarian cancer cells. Collagen was expressed surrounding tumor cells.

## Conclusions

Our results show that collagen expression is enhanced in ovarian cancer. A flaxseed diet reduces the presence of fibrosis in cancerous tissues and improves collagen isotropic structure. CB1 expression is increased in ovarian cancer. The upregulation of CB1 receptors coincides with the presence of inflammation and fibrosis. Our investigation supports that an association of the ECS and fibrosis is related to the onset of cancer. These data indicate that whole-flaxseed has anti-fibrotic properties and reduces fibrosis in the ovaries. We hypothesize flaxseed antifibrotic actions are mediated via modulation of the ECS. Future studies will examine the effect of flaxseed on CB1 expression, and use of second-harmonic generation imaging to quantify collagen arrangement.