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14. ABSTRACT
To gain better mechanistic insight of the role of Fifteen lipoxygenase-1 (15-LO-1) in prostate cancer (PCa), we recently developed a novel C57BL/6 transgenic mouse called 15-LO-1 in the Mouse Prostate (FLiMP). These mice, which conditionally express prostatic human 15-LO-1, display mouse prostatic intraepithelial neoplasia (mPIN) by week 20, but do not progress to cancer when on normal diet. Therefore, FLiMP mice provide an excellent model to study the experimental paradigm of PCa initiation, highlighting their usefulness in evaluating early "proactive" intervention strategies in PCa. In year 1, we have studied the effect of n-6 linoleic acid (LA) in diet on the onset of PIN in prostates of FLiMP+/+ mice and studies with n-3 Stearidonic acid (SDA) in diet is ongoing. We observed that, (a) wild type (WT) mice did not exhibit any prostate-specific phenotypic changes regardless of their diet, (b) FLiMP+/+ mice fed a diet high LA diet exhibited more aggressive PIN, with PIN-like changes observed in as early as 10 weeks compared to FLiMP+/+ mice fed a normal diet (PIN observed by week 20), and (c) the severity of these mPIN-like changes in the LA-diet fed mice are similar to those seen at 35 weeks or later in the FLiMP+/+ group which were on normal diet. This preliminary observation suggests the "bad" effects of excessive n-6 LA diet consumption in the progression of PCa.

15. SUBJECT TERMS Linoleic acid; LO or LOX, lipoxygenase; PUFA, Polyunsaturated fatty acid; PCa, Prostate Cancer, MMHCC, Mouse Models of Human Cancer Consortium; IHC, immunohistochemistry; H & E, Hematoxylin and Eosin; FLiMP, Fifteen Lipoxygenase-1 in the Mouse Prostate mouse model; DLP, dorsolateral prostate, VP, ventral prostate; AP, anterior

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INTRODUCTION:

Fifteen lipoxygenase-1 (15-LO-1) in the Mouse Prostate (FLiMP)- A novel mouse model to study impact of omega diets on prostate cancer progression: To gain better mechanistic insight of the role of 15-LO-1 in prostate cancer (PCa), we recently developed a novel C57BL/6 transgenic mouse called FLiMP [1]. These mice, which conditionally express prostatic human 15-LO-1, display mouse prostatic intraepithelial neoplasia (mPIN) by week 20, but do not progress to cancer when on normal diet. Therefore, FLiMP mice provide an excellent model to study the experimental paradigm of PCa initiation, highlighting their usefulness in evaluating early "proactive" intervention strategies in PCa. Our proposed studies are predicated on the hypothesis that dietary prevention is an effective means of eradicating PCa, and that FLiMP mice provide a valuable pre-clinical model for chemoprevention studies. Diets rich in either omega (n)-3 or n-6 polyunsaturated fatty acids (PUFAs) directly impact PCa tumor growth. Furthermore, the FLiMP mice, which overexpress human 15-LO-1, faithfully recapitulate the early stages of human PCa progression. These observations support the potential value of n-3 fatty acid SDA as a chemopreventative agent and the need for further studies. **Thus, the FLiMP mouse model has the strength of being a genetically defined, immune-competent tool to address the n-6 and n-3 experimental paradigm.**

BODY:

Task 1 in year 1. Assess the roles of dietary polyunsaturated fatty acid (PUFAs) diets in modulating the natural progression of PCa in the FLiMP$^{+/+}$ transgenic mouse model.

Experimental Approach:

In year 1, we have studied the effect of n-6 linoleic acid (LA) in diet on the onset of PIN in prostates of FLiMP$^{+/+}$ mice and studies with n-3 Stearidonic acid (SDA) in diet is ongoing. We utilized two isocaloric diets (4.4 kcal/gm) for our study; the n-6 i.e., LA containing diet and one normal chow. We initiated feeding the mice with high-LA and normal diets at week 6 and continued until the 22 weeks. Individual FLiMP $^{+/+}$ male mice on LA diet were sacrificed at 7, 10, 19, and 22 weeks of age, and examined for gross organ abnormalities. The urogenital tract was removed intact, and the tissue dissected to remove the bladder and associated fat. The tissue (including all prostate lobes, and the seminal vesicles) were fixed in buffered formalin. The urogenital tract, including the bladder, seminal vesicles, prostate (dissected dorsolateral, ventral and anterior), testes, and epididymes, were removed at necropsy *en bloc* and prepared for pathological evaluation. Prostate lesions were assessed according to the consensus classification of the Mouse Models of Human Cancer Consortium (MMHCC-October, 2000: Bar Harbor, Maine) by a pathologist (A. Parwani) blinded to the genotypes of the animals [2].

KEY RESEARCH ACCOMPLISHMENTS:

- **(1)** Wild type (WT) mice did not exhibit any prostate-specific phenotypic changes regardless of their diet.
- **(2)** Given that FLiMP$^{+/+}$ mice express 15-LO-1 and that the these enzymes convert n-6 LA to the pro-tumorigenic metabolite, 13-HODE, as expected, FLiMP$^{+/+}$ mice fed a diet high LA diet exhibited more aggressive PIN, with PIN-like changes observed in as early as 10 weeks compared to FLiMP$^{+/+}$ mice fed a normal diet (PIN observed by week 20).
- **(3)** The severity of these mPIN-like changes in the LA-diet fed mice are similar to those seen at 35 weeks or later in the FLiMP$^{+/+}$ group which were on normal diet.

REPORTABLE OUTCOMES: None.

CONCLUSION:

Importance and/or implications of the completed research: The observation of mPIN-like changes in the LA-diet fed FLiMP$^{+/+}$ mice as early as 10 weeks and mPIN occurring faster than those that were on normal diets suggests the "bad" effects of excessive n-6 LA diet consumption. These preliminary observations suggest the importance of n-6 PUFA in diets and their effect/s in the development of early PIN in the prostate. This could have been avoided by either limiting dietary use of excessive n-6 and/or include n-3 in the diets (by dietary chemoprevention). Although the dietary effects of n-3 on PIN development is in progress, we will need additional time for the experiment since we will be rederiving the mice to be able to achieve estimated numbers for statistical analyses. We are planning to rederive the mice from already frozen embryos from previous matings to avoid potential genetic drift and variability problems. The rederivation process will begin soon. All other planned experiments are on schedule.

REFERENCES:

1. Kelavkar, U.P., Parwani, A.V., Shappell, S.B. and Martin, W.D. (2006) Conditional expression of human 15-lipoxygenase-1 in mouse prostate induces prostatic intraepithelial neoplasia: the FLiMP mouse model. *Neoplasia*, 510-522.
2. Shappell, S.B., Thomas, G.V., Roberts, R.L., Herbert, R., Ittmann, M.M., Rubin, M.A., Humphrey, P.A., Sundberg, J.P., Rozengurt, N., Barrios, R., Ward, J.M. and Cardiff, R.D. (2004) Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. *Cancer Res*, **64**, 2270-305.

APPENDICES: None.

SUPPORTING DATA:
Histological Summary:

Table 1: Summary of Histopathological changes in the prostates of wild-type (WT) and FLiMP mice that were on normal diet.

Phenotype		WK7 (n=5)	WK14 (n=5)	WK21 (n=5)	WK24 (n=6)	WK35 (n=6)
FLiMP $^{+/+}$	NSPC (N)	3	2		3	
	HYPER (H)	1	2	1	2	+1
	mPIN LIKE FOCAL (MF)	3	1	4		2
	mPIN LIKE DIFF (MD)	-	-	1	3	3
WT	N	ND	4	1	1	2
	H	-	-	2	-	-
	MF	ND	ND	ND	ND	ND
	MD	ND	ND	ND	ND	ND

Table 2: Summary of Histopathological changes in the prostate of LA diet fed FLiMP mice.

	7 weeks (n=5)	10 weeks (n=5)	19 weeks (n=5)	22 weeks (n=6)
NSPC	1	-	-	-
Hyperplasia	4	2	1	1
Focal/mild mPIN-like changes including tufting and stratification	-	3	4	3+
Moderate to severe mPIN-like changes	ND	ND	ND	2*

*The severity of these mPIN-like changes are similar to seen at 35 weeks or later in the FLiMP$^{+/+}$ group (Table 1). NSPC:- no significantly pathological change. ND:- Not detectable.
+Mild atypical changes including nuclear crowding, stratification and tufting were seen as early as 10 weeks.

Histopathological Features

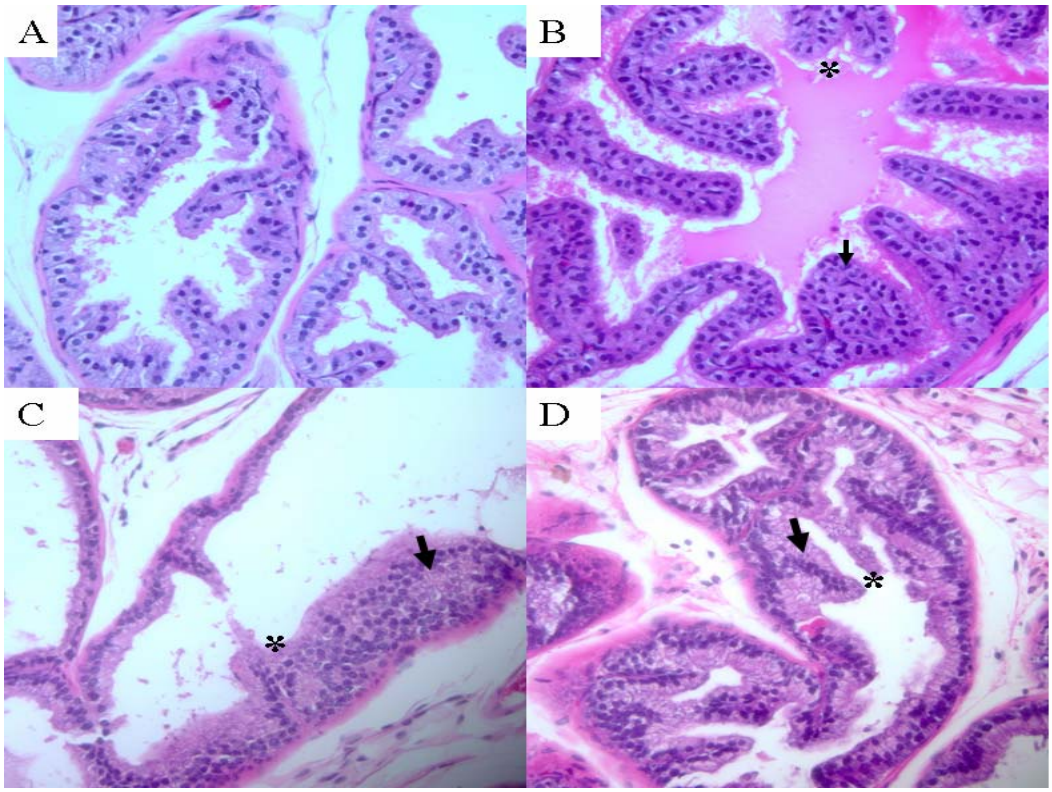

Figure 1: Histopathology of FLiMP mouse on normal and LA diets (**A-D**) showing a spectrum of pathological changes. H & E staining of paraffin sections of DLP in control (Normal diet) of 21 weeks of age (**A**), and LA diet fed animals (**B, C, D**) at only 10 weeks of age. Note that sections from **B, C and D** represent two different mice. Sections from the mice prostatic lobes demonstrate multilayered hyperplastic epithelium, with stratification of epithelial cells (Arrow). Focal tufting is observed (Asterisk). The luminal spaces are filled with eosinophilic secretions (Magnification is 400X). Taken together, the spectrum of histological changes seen in sections **B-D** resemble those seen in early or low grade human

intraepithelial neoplasia. These changes became more severe in older mice (refer to 22 weeks of age, Figure 2). No evidence of invasion was noted. In comparison, the prostate in control animal (not on LA diet) demonstrated similar changes but only at 21-22 weeks of age (Magnification is 200X).

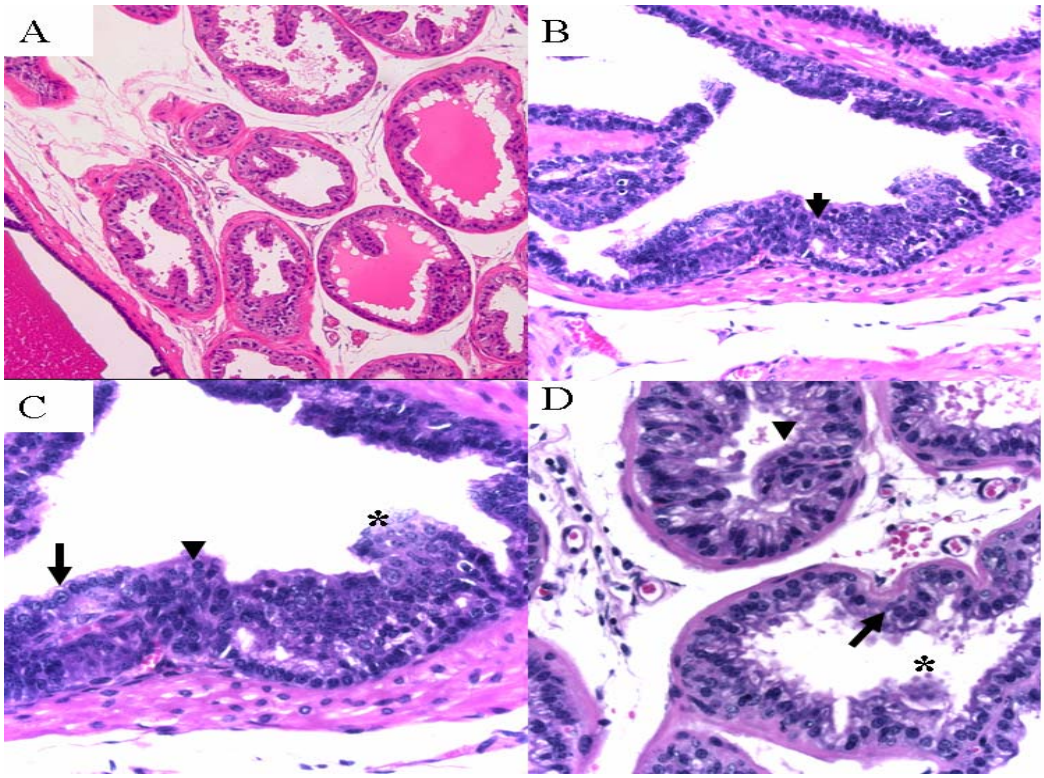

Figure 2: Histopathology of wild-type and FLiMP mice fed with LA diet (**A-D**) showing a spectrum of pathological changes. H & E staining of paraffin sections of DLP in wild type (A), and homozygous (**B, C, D**) mice at 22 weeks of age. Note that sections from B, C and D represent two different mice. Sections from the mice prostatic lobes demonstrate multilayered hyperplastic epithelium, with stratification of epithelial cells (Arrowhead). The epithelial cells demonstrate moderate to severe nuclear atypia (Arrow, **D**, Magnification is 400X). Focal tufting is observed (Asterisk). The luminal spaces are filled with eosinophilic secretions (Magnification is 400X). Taken together, the spectrum of histological changes seen in sections **B-D** resemble those seen in human prostatic intraepithelial neoplasia, satisfying the NCI MMHCC criteria for mPIN. No evidence of invasion was noted. In comparison, the wild type prostate demonstrates only mild epithelial hyperplasia (**A**) (Magnification is 200X).

Blinded histopathology analyses of dorsal prostate (DP) and lateral prostate (LP) sections of LA diet mice demonstrated focal epithelial stratification with generally mild nuclear atypia. Such lesions were observed in an increasing frequency of examined mice with age, and both their extent as well as architectural abnormalities was also noted to progress with age in the LA diet mice, satisfying the NCI MMHCC criteria for mouse prostatic intraepithelial neoplasia (mPIN). In the DP, tufting and micropapillary patterns of epithelial stratification with atypia were noted, corresponding to the most common architectural patterns of human HGPIN. The histopathologic alterations in the LP were the most prominent. Compared to the rather simple flat epithelium in wild type LP, LP sections from the LA diet animals showed progressively severe nuclear stratification, even achieving focal cribriform architecture (Table 2 and representative Figure 2B, C and D). Nuclear atypia was evident, with some enlargement and hyperchromasia, with chromatin clumping. No foci suspicious for invasive carcinoma were noted in any sections of the mice examined, and there were no changes noted in the stroma of FLIMP mice compared to wild type prostates. However, as proposed, we will need more mice to statistically conclude our observations and to publish the findings.