Impact of obesity on the uterine microbiome in pre- and postmenopausal mice with endometrial cancer

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INTRODUCTION

Endometrial cancer (EC) is the 4th most common cancer among women in the United States, and the frequency and mortality of EC continues to rise, in part, due to the obesity epidemic[1]. In 2020, approximately 65,620 new cases of EC will be diagnosed, and it is, on average, diagnosed in postmenopausal women[2]. Moreover, obesity is also associated with both higher risk of developing and dying from EC[3].

The *Lkb1$^{fl/fl}$p53$^{fl/fl}$* mouse has specific and somatic deletions of the tumor suppressor genes, *Lkb1* and *p53*, and is a transgenic mouse model of endometrioid endometrial cancer. Our prior studies have shown that obesity in *Lkb1$^{fl/fl}$p53$^{fl/fl}$* mice leads to more aggressive tumor behavior as demonstrated by a doubling of endometrial tumor size in obese as compared to lean mice. Additionally, we have found differences in genus-level microbiota in the tumors of obese *vs* lean mice as well as increased microbiome-associated metabolites in the tumors of obese mice.

The microbiome is thought to play a complex role in the regulation of obesity and cancer, yet the inter-relationship of obesity, the uterine tumor microbiota and EC pathogenesis is unknown, including the potential influence of menopausal status. Thus, we characterized the microbiota of the malignant uterus using pre- and postmenopausal, obese and lean genetically engineered mouse models of endometrioid EC (*Lkb1$^{fl/fl}$p53$^{fl/fl}$* mice).

METHODS

The *Lkb1$^{fl/fl}$p53$^{fl/fl}$* mouse is a genetically engineered, pre-clinical model of endometrioid EC. At 3 wks of age, *Lkb1$^{fl/fl}$p53$^{fl/fl}$* mice were fed a low fat diet (LFD, 10% calories from fat) versus a high fat diet (HFD, 60% calories from fat) to mimic diet-induced obesity. At 6 wks of age, the right uterine horn was injected with AdenoCre virus to delete *Lkb1* and *p53* and induce EC. Concurrently, mice either underwent bilateral oophorectomy to induce the postmenopausal state or retained their ovaries to maintain a premenopausal status. EC tumors were collected from all mice 12 wks after tumor induction. The microbiota profiles were characterized by bacterial 16S rRNA high throughput sequencing, and the data was analyzed using Qiime2 and MicrobiomeAnalyst.

RESULTS

Obese/HFD mice

	Premenopausal	Postmenopausal	p-values
Actinobacteria	0.03	2.14	0.095
Bacteroidetes	0.05	14.33	0.69
Deferribacteres	0	0.86	1
Firmicutes	0.07	44.15	0.056
OD1	0.01	0.1	0.047
Proteobacteria	0.86	38.39	0.151
TM7	0	0.03	0.007

Table 1: Phylum-level microbiota abundance (%) in obese mice with EC. There was a significant increase in the relative abundance of *OD1* and *TM7* in postmenopausal mice. There was a trend toward an increase in the abundance of *Firmicutes* in postmenopausal *vs* premenopausal mice.

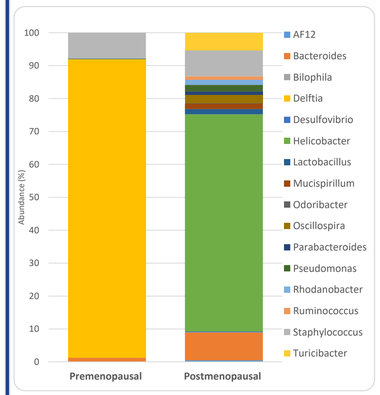

Fig 1: Genus-level microbiota abundance (%) in pre- *vs* postmenopausal obese mice with EC. See also Table 2.

	Premenopausal	Postmenopausal	p-values
AF12	0	0.46	1
Bacteroides	1.25	8.5	0.451
Bilophila	0	0.11	0.29
Delftia	90.68	0	0.007
Desulfovibrio	0	0.14	0.29
Helicobacter	0.02	66.01	0.01
Lactobacillus	0.13	1.59	0.672
Mucispirillum	0.03	1.66	0.131
Odoribacter	0	0.12	0.025
Oscillospira	0	2.61	0.007
Parabacteroides	0	0.92	1
Pseudomonas	0.11	2.02	0.914
Rhodanobacter	0	1.6	0.119
Ruminococcus	0	0.99	0.007
Staphylococcus	7.77	7.94	0.526
Turicibacter	0	5.33	0.29

Table 2: Genus-level microbiota abundance (%) in obese mice with EC. There was a significant increase in the abundance of *Delftia* in premenopausal mice whereas the abundance of *Helicobacter*, *Ordoribacter*, *Oscillospira* and *Ruminococcus* were increased in postmenopausal mice.

Lean/LFD mice

	Premenopausal	Postmenopausal	p-values
Actinobacteria	0.56	2.99	0.690
Bacteroidetes	2.02	13.45	0.056
Deferribacteres	7.76	0.92	1.000
Firmicutes	5.98	58.65	0.095
OD1	1.4	0.62	0.310
Proteobacteria	82.28	22.58	0.008
Thermi	0	0.41	0.607
Verrucomicrobia	0	0.38	0.290

Table 3: Phylum-level microbiota abundance (%) in lean mice with EC. There was a significant increase in the abundance of *Proteobacteria* in premenopausal mice. Also, there was a trend toward increased abundance of *Bacteroidetes* in postmenopausal mice.

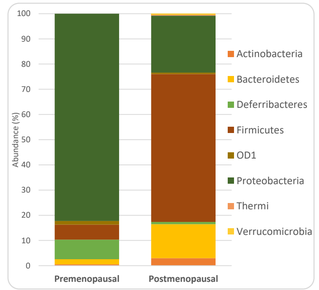

Fig 2: Phylum-level microbiota abundance (%) in pre- *vs* postmenopausal lean mice with EC. See also Table 3.

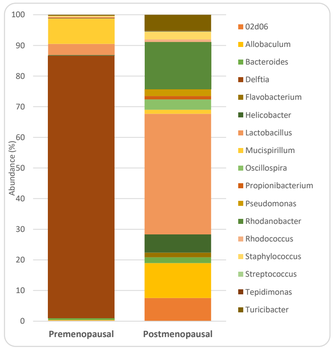

Fig 3: Genus-level microbiota abundance (%) in pre- *vs* postmenopausal lean mice with EC. See also Table 4.

	Premenopausal	Postmenopausal	p-values
02d06	0.21	7.55	0.451
Allobaculum	0.19	11.39	0.373
Bacteroides	0.62	1.9	0.824
Delftia	85.62	0	0.007
Flavobacterium	0.02	1.54	0.075
Helicobacter	0.23	5.96	0.480
Lactobacillus	3.61	39.35	0.530
Mucispirillum	8.18	1.31	0.824
Oscillospira	0.13	3.39	0.656
Propionibacterium	0.27	1.1	0.556
Pseudomonas	0.03	2.23	0.373
Rhodanobacter	0	15.4	0.119
Rhodococcus	0.22	0.85	0.373
Staphylococcus	0.37	2.48	0.139
Streptococcus	0	0.13	0.290
Tepidimonas	0.01	0.18	0.480
Turicibacter	0.29	5.25	0.526

Table 4: Genus-level microbiota abundance (%) in lean mice with EC. There was a significantly higher abundance of *Delftia* in premenopausal mice.

RESULTS

Postmenopausal mice

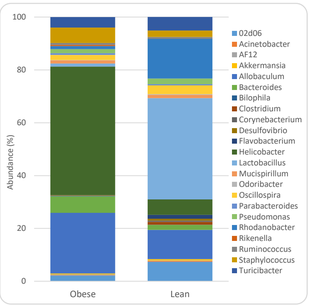

Fig 4: Genus-level microbiota abundance (%) in obese *vs* lean postmenopausal mice with EC.

	Obese	Lean	p-values
02d06	2.17	7.35	0.526
Acinetobacter	0.08	0.37	1
AF12	0.34	0.17	0.265
Akkermansia	0.39	0.49	0.666
Allobaculum	22.92	11.1	0.666
Bacteroides	6.26	1.86	0.106
Bilophila	0.08	0.07	0.914
Clostridium	0.06	1.09	1
Corynebacterium	0.13	0.47	0.29
Desulfovibrio	0.11	0.7	0.451
Flavobacterium	0.09	1.5	0.29
Helicobacter	48.65	5.81	0.018
Lactobacillus	1.17	38.35	0.421
Mucispirillum	1.22	1.28	0.091
Odoribacter	0.09	0.23	0.824
Oscillospira	1.92	3.3	0.094
Parabacteroides	0.68	0.45	0.746
Pseudomonas	1.49	2.17	0.6
Rhodanobacter	1.18	15	0.31
Rikenella	0.45	0.02	0.347
Ruminococcus	0.73	0.72	0.059
Staphylococcus	5.85	2.41	0.29
Turicibacter	3.93	5.12	0.917

Table 5: Genus-level microbiota abundance (%) in postmenopausal mice with EC. There was a significant increase in the relative abundance of *Helicobacter* in obese postmenopausal mice.

CONCLUSION

There were distinct differences in the bacterial composition of the ECs in *Lkb1$^{fl/fl}$p53$^{fl/fl}$* mice according to obesity and menopausal status. For example, the abundance of *Helicobacter* was significantly increased in obese, postmenopausal mice with EC whereas *Delftia* appears to dominate the microbiota composition of EC tumors of premenopausal mice regardless of obesity status. This data suggests that the microbiome may play a role in the pathogenesis of obesity-driven EC, particularly in postmenopausal patients.

Future cross-species comparisons could facilitate discovery of the underlying mechanisms of differing bacterial presence in EC tumors that occur based on obesity and menopausal status. We plan to continue ongoing research with the aim of defining the microbiota in endometrial cancers of obese and lean, pre- and postmenopausal women.

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