Compensatory alterations in energy homeostasis characterized in uterine tumors from hereditary leiomyomatosis and renal cell cancer

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Objective: To determine the molecular alterations that maintain energy homeostasis in hereditary leiomyomatosis and renal cell cancer (HLRCC) uterine tumors with disrupted fumarate hydratase, compared with nonsyndromic uterine tumors.
Design: Laboratory study.
Setting: Tertiary academic university hospital.
Patient(s): Eleven nonsyndromic leiomyoma–myometrium pairs and three HLRCC leiomyoma–myometrium pairs were obtained from patients who were recruited at national and military research centers in the United States.
Intervention(s): Molecular analysis.
Main Outcome Measure(s): Hereditary leiomyomatosis and renal cell cancer and nonsyndromic leiomyomas were compared with patient-matched myometrium for relative glycolysis and Krebs cycle gene expression.
Result(s): By microarray analysis, we confirmed that fumarate hydratase messenger RNA (mRNA) was underexpressed in HLRCC fibroids, compared with matched myometrium. Consistent with the possibility that alterations in fumarate hydratase represented a change to a more anaerobic state, we found that HLRCC fibroids overexpressed genes such as phosphofructokinase, aldolase, phosphoglycerate kinase, enolase, and pyruvate kinase. Expression of these genes was not altered in nonsyndromic leiomyomas. Furthermore, there were no overt changes in expression of Krebs cycle enzyme gene expression, with the exception of fumarate hydratase.
Conclusion(s): Our findings demonstrate that alterations in fumarate hydratase are compensated for by increases in glycolysis enzyme expression in HLRCC. (Fertil Steril® 2007;88(Suppl 2):1039–48. ©2007 by American Society for Reproductive Medicine.)

Key Words: Hereditary leiomyomatosis and renal cell cancer, fumarate hydratase, leiomyoma, glycolysis, enolase, aldolase, pyruvate kinase, Krebs cycle

The cumulative incidence rate of uterine leiomyomata by age 50 years is >60%, a rate that is higher than that of most significant maladies impacting women of reproductive age (1). Leiomyomas also are a common source of morbidity in women and are the most frequent reason that women seek hysterectomy (2). Despite this burden on both the patient and the health care system, there is limited understanding of the molecular causes of leiomyoma development. We (3–5) and other investigators (6–10) have identified, by microarray analysis, differentially expressed genes in benign leiomyomas compared with in myometrium. Microarray studies serve as a powerful screening tool for discovering genes that may differ between tissues. However, because of the large number of affected individuals combined with the large number of genes screened, it can be difficult to distinguish potential etiologic genes from genes that are specific to individual patients.

Familial studies assessing rare genetic syndromes with a high penetrance of leiomyomata provide an opportunity to identify specific etiologic genes. Vikhlyaeva and colleagues (11) demonstrated general evidence that fibroid development is genetically influenced. The familial syndrome hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal-dominant disease that carries a 75% rate of uterine leiomyoma development and an elevated rate of leiomyosarcoma development (12, 13). This

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syndrome also is associated with cutaneous leiomyoma and papillary renal cell cancer. Patients with the allele can develop large numbers of both uterine and skin leiomyomas. Given this pattern, Launonen and colleagues (13) suggested that the genetic disruption involves a tumor suppressor gene. By using genetic marker analysis, they isolated a likely etiologic gene to chromosome 1q42–q44. Prior studies (14, 15) demonstrated a linkage for the syndrome of multiple cutaneous leiomyomata and uterine fibroids (MCUL) to 1q42.3–q43. The close association between these similar syndromes led to a collaboration that resulted in the identification of decreased fumarate hydratase activity in patients with syndromes that generate leiomyomas (16, 17). Analysis of multiple families led to the identification of a large number of polymorphisms and mutations in the fumarate hydratase gene (18). Although the finding of fumarate hydratase alterations has been common, the mechanism by which fumarate hydratase may contribute to leiomyoma formation is more speculative. However, the role of fumarate hydratase as a tumor suppressor cannot be excluded.

Fumarate hydratase, an enzyme within the Krebs cycle, provides for aerobic metabolism. In fact, alterations in the subunits of a proximal enzyme in the Krebs cycle, succinate dehydrogenase, have been associated with renal cell carcinoma, pheochromocytoma, and paraganglioma (19, 20). These observations suggest that enzymes involved in glucose metabolism may play a role in altering the cellular phenotype. Analysis of energy homeostasis in HLRCC leiomyomata therefore was the next logical step.

In applying microarray analysis to HLRCC leiomyomata and patient-matched myometrium, we (21, 22) and other investigators (23) provided evidence that glycolysis gene mitochondrial RNAs (mRNAs) were disrupted in HLRCC. These results suggest that alterations in mRNA transcript levels may play a role in the HLRCC phenotype.

Tusher and colleagues (24) have generated statistical techniques that minimize the false-positive rate by using significance analysis of microarrays. However, any false-positive rate allows for the possibility that the alterations seen in glycolysis mRNA expression could occur by chance. We therefore evaluated the alterations in transcripts of glycolysis components by gene-specific techniques such as real-time reverse-transcriptase polymerase chain reaction (PCR), and we evaluated protein expression of key glycolysis and Krebs cycle enzymes, including fumarate hydratase.

The objective of this study was to determine compensatory alterations required to maintain energy homeostasis. We hypothesized that alterations that would disrupt the relative concentration or function of fumarate hydratase would have potentially deleterious effects on the conversion of glucose to adenosine triphosphate (ATP) via the Krebs cycle. One mechanism to compensate would be an increase in the level of anaerobic glucose metabolism. We therefore investigated the level of template and protein expression of key glycolysis and Krebs cycle enzymes.

MATERIALS AND METHODS

Study Population and Tissue Procurement

Three fibroid and myometrial tissue pairs were obtained from a patient with the HLRCC syndrome who was undergoing myomectomy. Surgery was performed at the National Institutes of Health. Eleven patients with nonsyndromic or common uterine fibroids consented and underwent hysterectomy at the National Naval Medical Center. Institutional review board approval was obtained. Tissue harvesting involved isolation of each fibroid as well as of adjacent myometrium. Approximately 3-mm^3 to 1-cm^3 tissue pieces were taken. For some fibroids, this represented the entire specimen. From larger fibroids, tissue was taken at random in the center, middle, and periphery. Patient characteristics are presented in Table 1.

Tissue destined for RNA isolation was minced and immediately placed in RNAlater (Ambion, Inc., Austin, TX). Samples were either immediately processed upon return to the laboratory or were placed at 4°C overnight and then placed in long-term storage at −70°C. Tissue destined for immunohistochemistry was cut to 5-mm^3 sections, placed in 10% formalin, and then stored at −70°C.

Isolation of RNA

Tissue (either fibroid or myometrial) was removed from the RNAlater solution and re-minced. Isolation of RNA was performed as described elsewhere (4). Integrity and concentration of RNA were confirmed by agarose electrophoresis and spectrophotometer (A260/A280 ratios).

Microarray Analysis

Samples of 15 mg of total RNA from fibroid and adjacent myometrium were provided to Capital Genomix (Rockville,

TABLE 1

Patient characteristics of spontaneous leiomyoma donors.

Patient no.	Age (y)	Race	Time of cycle[a]
1	43	AA	NA
2	35	C	Follicular
3	39	AA	Luteal
4	36	AA	NA
5	37	AA	Luteal
6	35	AA	Luteal
7	46	AA	Follicular
8	40	AA	NA
9	40	C	NA
10	36	C	Follicular
11	46	C	Luteal

Note: AA = African American; C = Caucasian; NA = patients on oral contraceptive pills or GnRH agonists.
[a] Phase of menstrual cycle.

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