[Frontiers in Bioscience E2, 882-905, June 1, 2010]

Mouse models of uterine corpus tumors: clinical significance and utility

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1. ABSTRACT

Uterine tumors, whether benign or malignant, are diagnosed in a significant portion of women and are associated with a number of co-morbidities that negatively impact quality of life. Uterine tumors can be derived from the epithelial (endometrial hyperplasia or carcinoma) and mesenchymal (leiomyoma, sarcoma) layers of the uterus. The exact etiologies of the various tumor types are yet to be defined. Collectively their development and progression often results from aberrant steroid hormone exposure or dysregulation of related growth factor signaling and apoptotic pathways, reflecting the role of steroid hormone-dependent signaling and survival pathways in the cycles of cell growth and involution that characterize normal uterine physiology. While molecular analyses of human tumors can identify candidate genetic and epigenetic lesions contributing to uterine tumor initiation and progression, *in vivo* genetic models are needed to establish the functional significance of such lesions and their contribution to tumorigenesis. For this purpose, genetically-engineered mouse models have proven valuable. Here we review genetically-modified mouse models that develop uterine tumors and compare their pathology, utility/feasibility, and discuss their clinical relevance.

2. INTRODUCTION

In vitro culture model systems can be used to elucidate the functional contributions of one or more gene products to normal or aberrant uterine biology. While well-established uterine/endometrial cell lines have proven useful in research, such *in vitro* models cannot faithfully replicate the many aspects of tumor progression that depend on tumor-host interactions. Variations among lines distributed among multiple laboratories amplify the underlying problem. Primary cultures, which have advantages for some types of studies, are less stable and often become refractory to treatment effects. This is due in part to an epithelial-mesenchymal transition in response to culture conditions. *In vitro* studies also tend to focus on a homogenous cell population that does not completely reflect the more heterogenous nature of primary tumors, which consist of tumor cells along with stromal, vascular and immune cellular components. Also of importance are the cell-cell interactions present in the tumor microenvironment, which are not typically replicated in a monolayer, collagen or agar base, or in spheroids. Nonetheless, *in vitro* studies have significantly advanced our knowledge of normal uterine biology (e.g. epithelial-stromal cell interaction, cell proliferation, differentiation, migration, adhesion, transformation and/or intra cellular signaling).

The caveats associated with cell culture have led many investigators to develop *in vivo* models. Consequently, numerous groups have developed and utilized mutant mouse models to better define the functional significance of gene products as they relate to the development, progression, invasive and/or metastatic potential of multiple cancer types. Our objective herein was to recognize and promote the significance of a subset of these mutant mouse models that display aberrant uterine growth as it relates to hyperplasia and/or tumor development (benign or malignant).

While researchers over the years have compiled long lists of oncogenes and/or tumor suppressors whose gain of function and/or loss have been associated with the onset, progression, chemoresistance and/or type of uterine tumors, there have been fewer studies to provide more convincing cause-and-effect relationships allowing us to realize the functional and pathological importance of the respective oncogenes and/or tumor suppressors. Here we have attempted to compile an in-depth list of mutant mouse models of uterine corpus tumors and discuss the insights they have yielded into the pathogenesis of endometrial hyperplasia as well as benign and malignant uterine tumors (Table 1). Tumors of the uterine cervix, which have a distinct etiology (human papilloma virus infection) are not discussed in this review.

3. ENDOMETRIAL HYPERPLASIA

Endometrial hyperplasia describes an increase in size of the endometrium resulting from an increase in its constituent components per unit volume (1). The incidence of endometrial hyperplasia in the general population is unknown, but it is diagnosed in approximately 5% of women being evaluated for postmenopausal bleeding (2, 3). Attention was first drawn to this entity by Thomas Cullen in 1900 when he observed that the most common type of endometrial cancer, endometrioid adenocarcinoma, frequently arises from areas of pre-existing hyperplasia. Factors strongly associated with endometrioid endometrial cancer such as obesity, polycystic ovarian syndrome (PCOS), nulliparity and unopposed use of estrogen are also associated with endometrial hyperplasia (4, 5). After years of research focused on endometrial hyperplasia, two facts became obvious; endometrial hyperplasia is a heterogeneous entity varying in its extent of glandular crowding, architectural disorganization and nuclear atypia (6-8), furthermore, the associated risk of endometrial cancer varies in accordance with the degree of complexity and atypia (9, 10).

Table 1. Summary of mutant mice with defective uterine phenotypes

Genotype	Treatment	Uterine Phenotype	Reference
PRKO	-	endometrial hyperplasia	(24)
PRAKO	Estrogen	Luminal Epithelium Hyperplasia	(25)
MISII-PTTG	-	Endometrial glandular hyperplasia	(26)
MMTV-EGF		Cystic Endometrial Hyperplasia	(28)
Mig-6$^{-/-}$	-	Endometrial hyperplasia	(37)
Brca1$^{FL/FL}$	-	Cervical squamous epithelial hyperplasia, endometrial hyperplasia	(43)
Brca1$^{S971A/S971A}$		endometrial hyperplasia	(45)
PR$^{cre/+}$*Ctnnb1*$^{f(ex3)/+}$, *PR*$^{cre/+}$ *Ctnnb1*$^{f/f}$	-	Endometrial glandular hyperplasia Squamous cell metaplasia	(54)
Tsp-1$^{-/-}$*/beta6*$^{-/-}$	-	Endometrial hyperplasia	(62)
dab2$^{+/-}$	-	Endometrial hyperplasia, endometrial adenocarcinoma	(77)
HMGA2	-	Endometrial hyperplasia	(85)
Pten$^{5+/-}$C57Bl6/129Sv/J	-	Endometrial complex atypical hyperplasia	(107)
Pten$^{4-5+/-}$	-	Endometrial atypical hyperplasia, non-invasive endometrial carcinoma	(109)
Pten$^{3-5+/-}$C57Bl6/129Jv	-	Endometrial atypical hyperplasia, endometrial carcinoma	(110)
Pten$^{+/-}$*/ Mlh1*$^{-/-}$	-	Neoplastic endometrial lesions	(112)
Pten$^{pr-/-}$*/p53*$^{pr-/-}$, *Pten*$^{pr-/-}$	-	Endometrial carcinoma	(114)
Pten$^{+/-}$*C57BL/6*	-	Endometrial atypical hyperplasia Endometrial adenocarcinoma	(115)
Cables $^{-/-}$	Estrogen	Endometrial adenocarcinoma	(118)
Lkb1$^{+/-}$	-	Endometrial adenocarcinoma	(122)
Par4 $^{-/-}$	-	Endometrial hyperplasia, endometrial adenocarcinoma	(135)
mTerc$^{-/-G5t}$	-	Endometrial intra-epithelial carcinoma resembling serous carcinoma	(142)
Amhr2$^{tm3(cre)Bhr}$/+;Ctnnb1^{tm1Mmt}/+		Myometrial hyperplasia	(149)
CaBP9k/Tag	-	Leiomyoma	(151)
mogp-Tag	-	Uterus, oviduct, vagina tumor	(123)
beta-actin $^{SVER/+}$/HS-*cre1*	-	Leiomyosarcoma	(152)
MMTV-CR-1	-	Leiomyosarcoma	(153)
LMP2$^{-/-}$	-	leiomyosarcoma	(157)
E2F-1$^{-/-}$	-	High grade sarcoma	(159)
p53$^{val135/wt}$	1,2-Dimethylhydrazine	Sarcoma	(160)
p53$^{+/-}$ CBA	*N*-ethyl-*N*-nitrosourea, Ethinylestradiol	Endometrial stromal sarcoma, endometrial hyperplasia, atypical endometrial glandular hyperplasia, endometrial polyps, endometrial adenocarcinoma	(161, 162)
MMTV beta*Trcp1*	-	Uterine tumor	(168)

Abbreviations used: PRKO: progesterone receptor knockout, PRAKO: progesterone receptor A knockout, MISII-PTTG: Müllerian inhibitory substance type II-pituitary tumor transforming gene, MMTV-EGF: mouse mammary tumor virus-epidermal growth factor, Mig-6: mitogen-inducible gene 6, Brca 1: breast cancer 1, Tsp-1/beta 6: thrombospondin-1/integrin beta 6, dab2: disabled-2, HMGA2: high-mobility group AT-hook 2, Pten: Phosphatase and tensin homologue deleted on chromosome ten, Cables: Cdk5 and Abl enzyme substrate, Lkb1: unknown, Par4: prostate apoptosis response 4, Terc: telomerase RNA component, Amhr2: anti Müllerian hormone receptor type 2, CaBP: calcium binding protein, mogp: murine oviduct-specific glycoprotein, beta-actin $^{SVER/+}$;HS-*cre*: beta-actin encoding simian virus large T antigen /cre driven heat shock promoter, CR-1: cripto-1, LMP2: low molecular mass polypeptide 2, E2F-1: E2F transcription factor 1, p53: protein 53, beta*Trcp1*: beta-transduction repeat-containing protein 1.

The endometrial cancer risk associated with different subtypes of endometrial hyperplasia was outlined by Kurman *et al* (11) as follows: simple hyperplasia (1%), complex hyperplasia (3%), simple hyperplasia with atypia (8%) and complex hyperplasia with atypia (29%) (11). Based on this study and others, the World Health Organization subsequently revised its classification in 1994. The new classification recognizes four categories of endometrial hyperplasia; (1) simple hyperplasia, (2) complex hyperplasia, (3) simple atypical hyperplasia and (4) complex atypical hyperplasia. However, some investigators have pointed out limitations in the above classification scheme. For these reasons, some investigators have supported the use of a two-tier classification scheme where lesions are either benign hyperplasia, and have a exceedingly low malignant potential as opposed to the endometrial intraepithelial neoplasia (EIN) lesions that have been associated with a higher propensity to progress to invasion. (12).

The progression of endometrial hyperplasia to an endometrial carcinoma which is commonly associated with long term unexposed estrogen exposure is one of the pathogenic mechanisms believed to play a part in the development of endometrioid endometrial adenocarcinoma. It has been documented in untreated hyperplasias that the risk of developing an endometrial carcinoma in the presence of atypical hyperplasia approaches 29% (11). More recent data from the Gynecologic Oncology Group has also demonstrated that up to 42% of women with an endometrial biopsy proven atypical endometrial hyperplasia will have a concurrent endometrial adenocarcinoma on hysterectomy specimen (13). Thus it is important to recognize which mouse models have evidence of endometrial hyperplasia and whether there are any clinical correlates that would support the notion that the gene/proteins of interest may contribute to early stage disease.

3.1. Models presenting with endometrial hyperplasia

3.1.1. Steroid hormone models

The relationship of steroid hormones and endometrial cancer are well recognized. Endometrial cancer is strongly associated with chronic exposure to unopposed estrogen (*i.e.*, obesity, estrogen-producing granulosa cell tumors, unopposed estrogen treatment)(14). Progestins have long been known to counter estrogen-induced cell proliferation and stimulate endometrial differentiation through its classic receptor (15).

The progesterone receptor (PR) is a member of a nuclear receptor superfamily of transcription factors. The amino-terminal of the PR molecule is its most hypervariable region in terms of size and sequences. This hypervariable region contains trans-activation functions that modulate both the level and promoter specificity of target gene activation (16, 17). In most target tissues, the PR is trans-activated by estrogen via the estrogen receptor (ER). This implies that some of the physiological responses attributed to the PR could conceivably stem from a combined effect of both estrogen and progesterone, rather than progesterone alone. It therefore became necessary to characterize the specific responses due to progesterone interaction with PR. Early attempts to purify the PR protein revealed that this receptor consists of two naturally occurring ligand binding isoforms termed PRA and PRB (18). These were subsequently shown to be derived from the same gene (16, 19).

The relevance of the PR to endometrial cancer was first reported by Kleine *et al* in 1982, (20) who reported xenografts of endometrial cancer in thymus aplastic mice grew slowly when PR positive if compared to their PR negative counterparts. Subsequently, another group using nude mice suggested that re-growth of known PR positive tumors after a period of tumoristasis correlated with PR down-regulation (21). However, most of the initial studies of PR were performed in relation to clinical endocrinology and basic molecular biology (16, 18, 19, 22, 23). While these studies have supported the role of progesterone as an essential steroid hormone for the establishment and maintenance of mammalian pregnancy, the significance of the receptor and its basic biology has only recently been realized.

3.1.1.1. Progesterone receptor knock-out (KO) (PRKO and PRAKO)

To better understand the distinct physiologic functions of the PR *in vivo*, a mutant mouse carrying a germline mutation in the PR locus was generated that targeted the PRA and PRB isoforms (PRKO) (24). The uteri from the hormonally treated PRKO female mutants were abnormally enlarged with fluid filled endometrial cavities. In addition, there was thickening of the uterine wall and proliferation of the mucosal and glandular epithelium. Interestingly, a second mutant was generated which lacked the progesterone receptor A isoform (PRAKO). Histologically, the epithelial morphology of control wild-type (WT), PRAKO and PRKO mice were reported to be similar (25). Furthermore all genotypes responded similarly to estrogen as evidenced by hyperplasia of the luminal epithelium. While the addition of estrogen and progesterone resulted in an inhibition of uterine epithelial proliferation in WT animals, the anti-proliferative effect of progesterone was absent in PRAKO. More interesting however, is the fact that treatment of PRAKO mice with estrogen in combination with progesterone resulted in a progesterone-dependent increase in uterine luminal epithelial cell proliferation compared to those PRAKO mice receiving estrogen alone. Thus, the PRAKO mouse model raises some interesting questions that should be incorporated into the thought process when utilizing progestins for treatment of endometrial hyperplasia or low grade endometrial cancer. One obvious issue is the classic notion that PRA counters the inhibitory effects of PRB. Conneely and colleagues suggested that the acquisition of a progesterone -dependent proliferative response indicated that PRA may diminish overall progesterone and estrogen responsiveness in the uterus (25). Thus it is important to consider specific progesterone receptor isoforms and how their ratio may influence outcome of hormone therapy.

3.1.2. Targeted oncogene models

3.1.2.1. Pituitary tumor transforming gene (PTTG)

PTTG is highly expressed in a number of human primary tumors suggesting that it may be involved in tumorigenesis. One of its major functions is the inhibition of sister chromatid separation during the cell cycle. El-Naggar *et al* (26) using the Müllerian inhibitory substance type II receptor (MISIIR) gene promoter directed PTTG expression to ovarian surface epithelium and pituitary to determine its function in ovarian epithelial cancer. The transgene expression was undetectable in the uterus. No visible tumor was observed upon gross examination of the ovary. Histologically, there were very few primary follicles, with an overall increase in corpus luteum mass which translated into an increase in ovary size in the transgenic mice. Histopathologic examination of the uteri revealed cystic glandular hyperplasia which is associated with sustained levels of estrogen acting on the uterus. These glands were cystically dilated, fluid filled and in some cases occupied the entire endometrium and displaced the stroma. There was an increase in mitotic cell count in the luminal and glandular epithelium when compared to WT counterparts. In the transgenic mice a 1.5 fold increase in the level of serum estrogen was observed when compared to wild-types, with no difference in progesterone levels. Luteinizing hormone levels and testosterone levels were elevated with no difference in follicle-stimulating hormone levels.

El-Naggar *et al* believe that by targeting PTTG to granulosa cells during the proliferative phase, it may promote follicle survival thereby enhancing follicular development and decreasing follicular apoptosis. This may result in an increase in the overall estrogen levels which would explain the development of cystic glandular hyperplasia observed in the PTTG transgenic mice. They concluded that PTTG is capable of inducing initial transformation but is not sufficient for tumorigenesis. Their reasoning was based on the lack of visible tumor in the PTTG over-expressed ovarian surface epithelium,

granulosa cells, and pituitary, but an increase in the size of the ovary, hyperplasia of the endometrium and an increase in luteinizing hormone and testosterone.

3.1.3. Epidermal growth factor receptor models

The ErbB receptor tyrosine kinase family has an important role in tumor etiology and progression. Over-expression of members of the epidermal growth factor receptor (EGFR) family (EGFR, HER2, ErbB3, ErB4) have a direct impact on cell proliferation, differentiation and migration, and are associated with various epithelial cancers (reviewed by (27)). As a result these receptors have been intensely studied to understand their importance in cancer and as targets for directed therapy.

3.1.3.1. EGFR

To address whether EGFR has a role in the onset of reproductive cancers, Marozkina *et al* (28) generated transgenic mice with expression of EGFR driven by a mouse mammary tumor virus (MMTV) promoter. Mice were housed for 1.7 - 2 years after which time tissues were harvested for histopathological examination. 8/9 female mice presented with varying degrees of cystic endometrial hyperplasia described as mild to moderate when compared to age matched WT controls. Two other EGFR transgenic mouse models generated by Brandt and colleagues (29) had no uterine phenotype. Marozkina *et al* (28) hypothesized that introduction of EGFR alone can result in pre-neoplastic changes, specifically in the uteri and ovaries. However, secondary hits are needed for tumor onset and progression.

3.1.3.2. MIG-6

Mitogen-inducible gene 6 (*Mig-6*) is also known as ErbB receptor feedback inhibitor 1 (*Errfi1*), receptor-associated late transducer (RALT), or gene 33 (30-32). Mig-6 inhibits EGF signaling by interfering with the kinase domains of EGFR and ErbB2 (33-36). *Errfi1*$^{-/-}$ mice (no Mig-6) have been found to have EGF receptors that are hyperactivated and are known to develop hyperplasia or tumors in the skin, lung, or biliary system (30, 36). Therefore, several studies have proposed that Mig-6 acts as a tumor suppressor (30, 36).

To further characterize Mig-6, Jin *et al* generated *Mig-6* mutant mice (37). Since mice with homozygous loss of Mig-6 either are lost as embryos or have severe tissue deformities with a shortened life expectancy on average, these authors generated a Mig-6 conditionally mutated mouse. The group bred *Mig-6* heterozygous mice and obtained 14 *Mig-6*$^{-/-}$ female mice. 2 of these mice survived to the age of 9 months, and it was observed that their uteri were markedly larger compared to their WT counterparts. On microscopic examination, it was found that these *Mig-6*$^{-/-}$ females had endometrial hyperplasia.

3.1.4. Genetic alterations associated with endometrial hyperplasia

Certain hereditary factors predispose women to reproductive cancers and are associated with specific types of tumor. One of the most recognized of these, at least in terms of pre-disposition of early-onset breast and ovarian cancers are mutations in the BRCA1 gene, while mutations in beta-catenin are associated with type I endometrial cancers.

3.1.4.1. BRCA1

BRCA1 encodes a full-length BRCA1 protein (BRCA1-FL) as well as two smaller size protein products, BRCA1-delta11 and BRCA1-IRIS, due to alternative splicing. BRCA1-delta11 arises from in-frame splicing between exon 10 and 12 (38, 39). Although classically *BRCA1* mutations have been linked to breast and ovarian carcinomas there is conflicting evidence that *BRCA1* mutations may play a role in endometrial tumorigenesis. Niederacher *et al* (40) investigated 113 primary endometrial cancer samples and reported loss of heterozygosity (LOH) for *BRCA1* in 18.1%. They also discovered that LOH for *BRCA1* correlated with medium grade, positive ER status, family history of cancer and decreased overall survival. However, Levine *et al* (41) examined a cohort of 199 Ashkenazi Jewish patients with endometrial cancer and found only 3 patients with germline *BRCA* mutations (1 in BRCA1 and 2 in BRCA2). While BRCA1/2 carriers do have a slightly elevated risk, it is attributed to the tamoxifen treatment as a result of diagnosis of previous breast cancer (42). Thus while it is possible that germline BRCA mutations contribute to endometrial cancer the contribution appears to be minor.

In order to address the potential functions of BRCA1delta11 Kim *et al* (43) employed a cDNA knock-in approach that specifically blocked alternative splicing from exon 10 to exon 12, therefore creating a mice (*Brca1*$^{FL/FL}$) with intact BRCA1-FL and deleted BRCA1-delta11 protein. Using this model, the group demonstrated that this deletion did not interfere with the normal development or the responsiveness to acute DNA damage by γ-irradiation. These mice also displayed no obvious defects in any cell cycle checkpoints analyzed. The *in vivo* and *in vitro* data suggested that prolonged effects of BRCA1-delta11 deficiency resulted in increased expression of cyclin A and cyclin E, centrosome amplification, and faster G_1/S transition. *Brca1*$^{FL/FL}$ mice exhibited hyperplasia in the mammary gland, ovary, cervix, and endometrium and were prone to spontaneous tumor formation. Of the 10 *Brca1*$^{FL/FL}$ female mice, which were examined between 13 to 23 months of age, 5 mutant mice developed endometrial hyperplasia.

Kim *et al* (43) believe that accumulation of cyclin E in the mutant mice may play a part in the occurrence of mammary gland and reproductive tract hyperplasia. Previous studies have documented a correlation of mammary gland hyperplasia and carcinoma in overly expressed cyclin E transgenic mice (44). Notably cyclin E was accumulated on the epithelial cells of the hyperplastic ducts in the *Brca1*$^{FL/FL}$ mice.

A second model by Kim *et al* (45) focused on a mutation of the Chk2 phosphorylation site in BRCA1. During DNA damage response, BRCA1 is phosphorylated by several protein kinases including ATM, ATR, CHK1, CHK2, and MDC1. It has previously been shown that somatic mutations of CHK2 have been found in carcinomas

of the breast (46), colon (47), lung (48), and vulva (49). CHK2 is believed to be involved by modulating BRCA1 functions through phosphorylating BRCA1 after DNA damage. This phosphorylation of BRCA1 at S988 by CHK2 is required for dissociation and relocalization of BRCA1 which is important for cell survival after DNA damage (50).

To test the influence of phosphorylation of CHK2 on BRCA1 function *in vivo,* mice were generated that contained a mutation at the CHK2 phosphorylation site (S971). These mutant mice (*Brca1*$^{S971A/S971A}$) developed normally and survived for 1.5 years without obvious abnormalities. Disruption of BRCA1 phosphorylation increased the incidence of tumor formation. 4/6 *Brca1*$^{S971A/S971A}$ mice examined at approximately 2 years of age had dense branches in mammary glands with hyperplastic foci. 7/8 *Brca1*$^{S971A/S971A}$ females showed enlarged uteri and 3 lost their ovaries with blood aggregates, while the ovaries of the remaining exhibited abnormal structures. Tumor formation (lymphoma, mammary, colon and uterine) was also increased by administering a DNA-damaging agent, 1-methyl-1-nitrosurea, as well as with γ-irradiation.

3.1.4.2. Beta –catenin

Beta-catenin, a component of the Wnt signaling pathway involved in cell differentiation, is important for normal uterine function (51). Mutations in beta-catenin are a characteristic of endometrioid-type (type I) endometrial cancers (52, 53). Jeong *et al* (54) generated transgenic mice in which beta-catenin was stabilized (*PR*$^{cre/+}$ *Ctnnb1*$^{f(Ex3)/+}$) and conditionally ablated in the uterus (PR$^{cre/+}$ Ctnnb1$^{f/f}$). *PR*$^{cre/+}$ *Ctnnb1*$^{f(Ex3)/+}$ female mice were sub-fertile while *PR*$^{cre/+}$ *Ctnnb1*$^{f/f}$ were infertile demonstrating that altering beta-catenin expression in PR-expressing cells has a negative fertility effect. Using both models the group showed that alteration of beta-catenin affects uterine morphology. By 8 weeks both mouse models had smaller uteri than age matched controls. Clinically, it should be noted that the beta-catenin pathway has been implicated in squamous metaplasia and squamous differentiation in endometrioid endometrial cancers (54-56).

Histologically, differences in uterine morphology, as characterized by enlarged glands, were evident in mice which had stabilized beta-catenin by 2 weeks of age. At 6 weeks evidence of endometrial hyperplasia was present, with an increase in gland size, number and presence of hyperchromatic nuclei. Cyclin D1 a target of beta-catenin was increased in the glandular epithelium and not stroma. Estrogen receptor alpha (ER alpha) expression levels were also increased in the epithelium with reduced stromal levels. In contrast, mice in which beta-catenin was ablated, had fewer glands, with squamous cell metaplasia in the luminal epithelium, no alteration of cyclin D1 in glandular epithelium and increased ER alpha levels in the basal epithelial cells only. Thus, the authors demonstrated that correct regulation of beta-catenin is important to maintain normal uterine function.

3.1.5. Aberrations in matricellular proteins, phosphoproteins and hyperplasia

Matricellular proteins are key modulators of cell-matrix interactions and cellular functions. Their up-regulation has been documented in various tumors but little is known about their functions in tumor growth, survival, and metastasis. Aberrations in such proteins and in phosphoprotein signaling responses may lead to neoplastic transformation and ultimately cancer onset.

3.1.5.1. Thrombospondin-1/Integrin beta6

Thrombospondin-1 (TSP-1) is a matricellular protein involved in tissue remodeling associated with wound healing, neoplasia and embryonic development (57). In tumorigenesis TSP-1 suppresses angiogenesis by inhibition of endothelial cell proliferation and by activation of transforming growth factor beta (TGF beta)(57). Several studies have outlined the importance of this protein in tumor progression where its expression was reported as being inversely related to tumorigenesis (bladder) (58) and to survival rate (breast, lung, and colon carcinomas) (59-61). Ludlow *et al* (62) initially developed a TSP-1^{null} mouse to investigate lung disease. In their study TSP-1^{null} mice were crossed with mice deficient for epithelial specific alpha v beta6 integrin (referred herein as beta6). The presence of this integrin was shown previously to correlate with poor prognosis in gastric cancer (63) and lung cancer (64). In addition increases in its protein expression level is associated with clinical grade of ovarian cancer (65). In the Ludlow study the majority of TSP-1^{null}/beta6^{null} were viable. A variety of tumors spontaneously developed in these mice, including gastric, lung and colon. Endometrial hyperplasia was reported in 38% of double mutants, squamous cell carcinoma of the cervix and vagina was evident in 27%, and there was one incidence of leiomyoma (4%). WT and TSP-1^{null} mice did not develop any evidence of uterine abnormalities, with the exception of beta6^{null} mice which developed squamous cell carcinoma of the cervix and vagina. Interestingly, Behera and colleagues (66) concluded in their studies of human uterine fibroids that under expression of TSP-1 may contribute to their overall development. While the TSP-1^{null}/beta6^{null} mice had evidence of endometrial hyperplasia, over-expression of alpha v beta6 integrin has been reported in endometrial cancer and squamous carcinoma of the cervix (67, 68). Moreover, antibodies directed at integrin alpha v beta 6 have been shown to inhibit tumor progression (69). Thus the significance of the double mutant as it pertains to the human disease is not readily obvious.

3.1.5.2. Disabled-2

Disabled-2 (Dab2), a phosphoglycoprotein involved in cellular signal transduction, was initially identified as a putative tumor suppressor from analyses of differentially expressed transcripts in ovarian carcinomas (70, 71). Loss of protein expression is documented in a variety of cancers including ovary (71, 72), breast (73), bladder (74), pancreatic metastases (75) and colon (76). To further elucidate the role of Dab2 deficiency in tumor development, Yang *et al* (77), generated *dab2*$^{+/-}$ mouse colonies. At 6 months of age female *dab2*$^{+/-}$ are essentially

sterile. Histological analyses of the uteri from these mice revealed widespread luminal endometrial hyperplasia and dysplasia. Yang et al characterized their findings in terms of presence of endometrial hyperplasia (grade I-IV) and/or early endometrioid adenocarcinoma (grade IV with myometrial invasion). Grade I (hyperplasia without atypia) presented in 13/134 of dab2+/-, grade II (mild atypical hyperplasia) in 25/134, grade III (moderate atypical hyperplasia) in 8/134 and grade IV (complex atypical hyperplasia) in 2/134. From 33 dab2+/+ controls, 1/33 presented with grade I hyperplasia.

The authors proceeded to investigate Dab2 protein expression in 3 human benign endometrial and 28 high grade endometrioid carcinoma formalin fixed paraffin embedded tissue blocks. Dab2 staining was absent in all of the carcinoma cancer cells, but present in normal cells. Additionally, Dab2 expression by Western blot analyses was performed on a variety of endometrial and cervical cell lines. Dab2 expression was not detected in 4/6 endometrial carcinoma lines and 4/8 cervical carcinoma lines. Other phenotypes associated with the *dab2*$^{+/-}$ mice include ovarian, prostate and skin abnormalities. As a small fraction of *dab2*$^{+/-}$ mice presented with endometrial carcinoma, the authors postulate that Dab2 may be a contributing factor, rather than a causative one, in the onset of uterine tumors.

3.1.5.3. HMGA2

The high-mobility group AT-hook 2 (HMGA2) is a member of the high-mobility group AT-hook family proteins (78). These proteins are involved in transcription at specific target promoters by increasing the DNA-binding affinity of transcription factors (79). HMGA2 is almost exclusively found in undifferentiated mesenchyme (80). It has been linked to many benign mesenchymal tumors including lipomas (81), fibroadenomas of the breast (82), salivary gland adenomas (83), and endometrial polyps as a frequent target of chromosomal translocations (84).

Zaidi *et al* (85) used a transgenic mouse model to answer the question whether miss-expression of full-length, truncated, or both versions of the HMGA2 transcripts under a differentiated-mesenchyme-specific promoter would give rise to mesenchymal tumors. This group found there was a population of transgene-expressing tissues which exhibited a neoplastic phenotype, including the uterus. Others included the breast, salivary gland and preputial gland. Of interest to this review they found that 3 of 4 females exhibited overgrowth of the uterus and on histologic examination, these tissues showed endometrial hyperplasia.

They also found by IHC that HMGA2 expression in the neoplastic tissues of the HMGA2-transgenic mice was expressed only in the stromal component of the breast fibroadenomas, salivary gland adenomas, and preputial gland hyperplasias, whereas the epithelial component were negative for HMGA2 expression. There was no comment made on HMGA2 expression in the uterus. In using this model to extrapolate to humans it is important to note that the tumor types found in the transgenic mice mimic many of the tumor types that are observed in humans that have recurrent rearrangements involving the HMGA2 gene.

4. ENDOMETRIAL CANCER

Most uterine corpus tumors are of epithelial origin and include tumors of diverse histologic subtypes including endometrial endometrioid carcinoma, serous carcinoma, malignant mixed Müllerian tumor (carcinosarcoma), and clear cell carcinoma (Figure 1). Mesenchymal tumors of the uterus include, but are not limited to, benign leiomyoma and malignant leiomyosarcoma and endometrial stromal sarcoma.

Endometrial cancers are often classified into two major divisions (types I and II) (86) based on histopathology, clinical outcome, and epidemiology. The majority of sporadic endometrial carcinomas (~80%), designated as type I, occur predominantly in pre- and peri-menopausal women under unopposed estrogen stimulation and are often preceded by endometrial hyperplasia. Histologically, the majority of these tumors are designated as low grade endometrioid carcinomas (14, 87) and are characterized by, but not limited to, genetic alterations such as microsatellite instability (MSI) (88, 89), *PTEN* (90-93), *KRAS* (94, 95) and *beta-catenin* (52) mutations. In contrast, type II endometrial cancers generally occur in post-menopausal women, are estrogen independent and are pre-dominantly high grade serous carcinoma, malignant mixed Müllerian tumor, or clear cell carcinoma by histology (87). A subset of the grade 3 endometrioid carcinomas can also be considered as type II tumors. The type II carcinomas are associated with an aggressive clinical course and poor prognosis. Unlike endometrioid carcinomas, MSI, *PTEN* and *KRAS* mutations are rare in type II carcinomas with *p53* mutations (90, 96), *p16* inactivation (97), increased Her2/neu amplification (98, 99) and LOH on various chromosomes (100) being more frequent.

4.1. Mouse models presenting with endometrial carcinoma

4.1.1. Genetic alterations associated with endometrial cancers

4.1.1.1. PTEN and Type I endometrial carcinoma

PTEN is best known for its regulatory role in the insulin/insulin like growth factor pathway and as a tumor suppressor. It acts as a phosphatase to facilitate dephosphorylation of phosphatidylinositol (3, 4, 5) - triphosphate. Loss of PTEN function results in elevated levels of phosphatidylinositol (3, 4, 5) - triphosphate stimulating the AKT (also known as protein kinase B (PKB) signaling cascade) leading to sustained cell proliferation, enhanced cell survival and inhibition of apoptosis. PTEN can be inactivated by several mechanisms such as mutation, LOH and promoter hypermethylation.

The most prevalent genetic alteration of *PTEN* in endometrial cancers is mutation, which often coexists with LOH. *PTEN* mutations have been reported in up to 55% of endometrial hyperplasias (101, 102) and in approximately 83% of endometrial carcinomas that were preceded by a premalignant stage (101), suggesting that loss of PTEN function represents an early event in endometrial carcinogenesis. LOH without mutation, although less

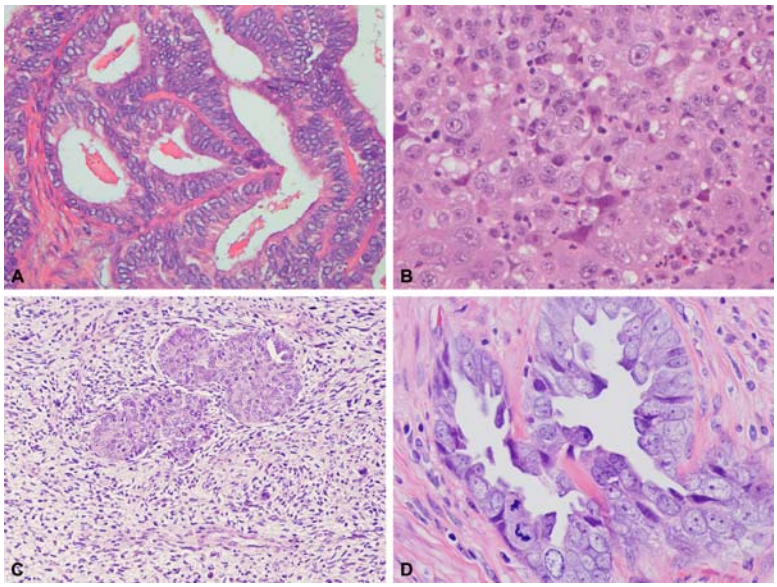

Figure 1. Pathology of human endometrial carcinoma. Endometrial carcinomas are broadly classified as endometrioid (A and B) and non-endometrioid (C and D). The non-endometrioid carcinomas are typically associated with advanced clinical stage and poor prognosis. Endometrioid carcinomas are graded 1-3, based on the percentage of glandular and solid components microscopically. Well-differentiated endometrioid tumors (grade 1) are composed mainly of well-formed neoplastic glands (A), while poorly differentiated endometrioid tumors (grade 3) are composed mainly of solid sheets of tumor cells, with only occasional gland formation (B). Non-endometrioid carcinomas include malignant mixed Müllerian tumor (C), uterine serous carcinoma (D), and clear cell carcinoma (not shown). Malignant mixed Müllerian tumors are composed microscopically of carcinomatous elements and sarcomatous elements. Serous carcinoma is characterized by papillary architecture and extreme cytological and nuclear atypia.

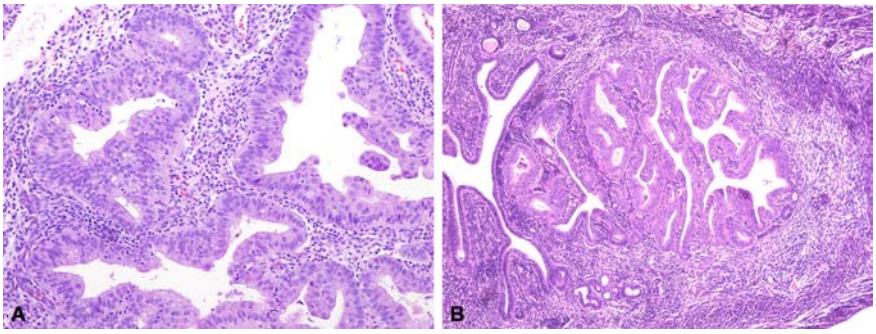

Figure 2. Photomicrographs of human (A) and PTEN heterozygous mouse (B) endometrial hyperplasia. (A) Human endometrial complex hyperplasia with atypia. Note the proliferation of enlarged endometrial glands with intervening stroma. The presence of intervening stroma helps to distinguish endometrial hyperplasia from grade 1 endometrioid adenocarcinoma. (B) PTEN heterozygous mouse endometrial hyperplasia. Note that the hyperplastic lesion resembles the human counterpart and is confined to the endometrial layer. The endometrial hyperplasia is not diffuse, as normal endometrium is also present. Hyperplasia in humans can also be localized rather than diffusely involving the endometrial cavity.

frequent than mutation alone, still occurs in approximately 40% of type I carcinomas (103, 104). *PTEN* mutations have been reported in 60–86% of MSI-positive endometrioid but at a lower incidence (24–35%) of MSI-negative tumors (91, 93, 105, 106). Finally, *PTEN* promoter methylation with altered PTEN has been found in ~20% of advanced stage endometrial carcinomas (93). Thus recognizing PTEN mutations are the most common genetic alteration in type I endometrioid carcinomas (90) it is not surprising that there have been significant biologic functional contributions made from the development of PTEN mutants (Figure 2).

4.1.2. Pten Models

4.1.2.1. Non tissue specific deletions

PTEN deficient mice have been generated by various groups (107-109) in order to understand the functional significance of *Pten* inactivation in tumorigenesis. *Pten*$^{-/-}$ mutant mice are embryonic lethal (E6.5-E9.5) (107, 108) resulting from abnormal development *in utero* (108). Additionally, mouse embryonic fibroblasts (MEFs) derived from *Pten*$^{-/-}$ embryos show decreased sensitivity to apoptotic stimuli, including UV irradiation, heat shock, osmotic stress, and tumor

necrosis factor alpha stimulation. *Pten*$^{-/-}$ MEFs display constitutively elevated activity of PKB/Akt, which can be restored to normal levels by the re-introduction of PTEN. Consistent with this, an active mutant of PKB/Akt can suppress apoptosis induced by *Pten* over-expression (110). This suggests that PTEN absence may contribute to tumorigenesis by either increased cellular proliferation rates and/or by protection against apoptosis.

The usefulness of *Pten* mouse models in the study of tumorigenesis is readily apparent by the predisposition of *Pten*$^{+/-}$ mice to form a wide variety of cancers including endometrial, breast, prostate and lymphomas. Significant differences in tumor spectra in various studies have been attributed to genetic background. *Pten*$^{+/-}$ mice on a C57Bl/6 x 129Sv background have a higher tendency to develop endometrial carcinomas than those on a CD1 background (108, 110). DiCristofano *et al* (109) reported no uterine malignancies on deletion of *Pten* exons 4-5 in mice on the inbred C57Bl/6 background. But a variety of other carcinomas (colon, thyroid) and hyperplasias/dysplasias (prostate, colon and skin) developed at <3.5 month of age. The tumors noted in the *Pten*$^{+/-}$ model developed by Suzuki *et al* (108) differed somewhat from that of the DiCristofano *et al* model (109). Suzuki *et al*, using mice on the outbred CD1 background with *Pten* exons 3-5 deleted, monitored heterozygous mice for ~28 weeks. Although tumors developed in 14% of *Pten*$^{+/-}$ mice and no tumor development was reported in *Pten*$^{+/+}$ mice, similar to DiCristofano's findings, no uterine malignancies occurred. Tumors were confined to T-cell lymphomas, atypical adenomatous hyperplasia of the liver and teratocarcinoma.

A more recent study from this group utilized *Pten*$^{+/-}$ mice from the inbred C57Bl/6 and 129Jv backgrounds (110). In this case *Pten*$^{+/-}$ female mice were monitored for tumor formation for a 26-65 week period. All females, 26 weeks or older, showed endometrial hyperplasia with high grade atypical hyperplasia more common in mice older than 30 weeks than in younger mice (45 v 14%). Similar to clinical evidence where mutations of *Pten* have been reported in all endometrial neoplasia stages (90-92), this group reported LOH at the *Pten* locus in all stages of hyperplasia. The incidence of endometrial carcinoma was 14% in the 30 week and older age bracket, however no age correlation was found. As with earlier work on *Pten*$^{-/-}$ MEFs (110), *Pten*$^{+/-}$ associated endometrial carcinomas were found to have hyperphosphorylated PKB/Akt. Additional abnormalities reported were classified as Dunn's adenocarcinoma and fibroadenoma (breast), medullary hyperplasia, gastrointestinal dysplasia and lymphoma. At 3 months of age it was reported that 100% of *Pten*$^{+/-}$ female mice on a pure 129/Sv background spontaneously develop endometrial hyperplasia, which progressed to *in situ* carcinoma by 5-9 months in ~30% of mice (109). By 10 months of age the majority of these mice succumb to hemorrhage and necrosis arising from destruction of the uterine horns. Another group deleted *Pten* exon 5 to generate *Pten*$^{+/-}$ mice on the C57Bl/6 and 129Sv background reported endometrial hyperplasia (100%), lymphomas, dysplastic intestinal polyps, prostate and thyroid neoplasias at mean age of 26.8 weeks (107).

4.1.2.2. Pten$^{+/-}$/ Mlh1$^{-/-}$ Model

MLH1 encodes a protein involved in DNA mismatch repair and loss of its expression is associated with MSI-positive endometrioid carcinomas (111). Wang *et al* (112) investigating the association of *Pten* mutations and DNA mismatch repair deficiency (detected as MSI) generated a *Pten*$^{+/-}$/ *Mlh1*$^{-/-}$ double mutant mouse. All of *Pten*$^{+/-}$/ *Mlh1*$^{-/-}$ mice developed endometrial lesions, including invasive carcinoma similar to that developed by the *Pten*$^{+/-}$ mice at a similar time point (18 weeks), with LOH frequency similar to that observed in older *Pten*$^{+/-}$ mutants (40 weeks), suggesting that DNA mismatch repair deficiency can accelerate endometrial tumorigenesis in *Pten* $^{+/-}$ mice. Importantly the authors reported these lesions were morphologically similar to that observed in human endometrial carcinomas.

4.1.2.3. Targeted endometrial Pten deletion

To overcome the early embryonic lethality caused by conventional knockout strategies and to gain a more targeted approach to the study of tumorigenesis, tissue specific conditional deletions of *Pten* have been used in the study of prostate, mammary, lung, ovary and brain tumors. Until recently there were no tissue specific conditional KO mouse model systems for studying endometrial cancer although tumor incidence and type in *Pten*–deficient mice were assessed by generation of a Cre/LoxP system to excise *Pten* exon 5 in a controlled manner by the administration of 4-OHT (113). This model has the advantage of rescuing embryonic lethality due to complete *Pten* inactivation. Endometrial carcinoma was reported as occurring in 46% of mice age 7-9 week post 4-OHT treatment.

Daikoku *et al* (114), were the first group to use a conditional deletion of *Pten* in the endometrium by crossing floxed *Pten* mice with mice expressing Cre under the regulation of the progesterone receptor promoter (*Pten*$^{pr-/-}$). Additionally, endometrium specific *Pten* and/or *p53* (*Pten*$^{pr-/-}$/*p53*$^{pr-/-}$) mutants were developed using the same strategy. All *Pten*$^{pr-/-}$ mice developed endometrial hyperplasia at ~3 weeks while *Pten*$^{pr-/-}$/*p53*$^{pr-/-}$ double mutants developed non-invasive endometrial carcinoma by the same time period. By 3 months *Pten*$^{pr-/-}$ endometrial hyperplasia had progressed into *in situ* carcinoma while *Pten*$^{pr-/-}$/*p53*$^{pr-/-}$ mutants had evidence of myometrial invasion. Control *Pten*$^{pr+/+}$ and *Pten*$^{pr+/+}$/*p53*$^{pr+/+}$ showed no evidence of abnormal uterine pathology up to 5 months of age, nor did *p53*$^{pr-/-}$ mice. It was also noted that the double mutants had an advanced mortality rate.

4.1.2.4. Pten loss and steroid response

Although type I endometrial cancers arise under unopposed estrogen stimulation, *Pten*$^{+/-}$ mice have normal estrous cycles, hormone serum levels and remain fertile. Using the 129/Sv *Pten*$^{+/-}$ model Vilgelm *et al* (109) have demonstrated that PTEN loss in the mouse endometrium results in activated Akt leading to increased ER alpha phosphorylation and subsequent activation of ER dependent pathways. ER alpha activation as a consequence of PTEN loss is clinically relevant as type I endometrial carcinomas typically have elevated expression levels of ER

alpha (104). Similar to previous studies (107, 109, 115). Daikoku *et al* (114) demonstrated an increase in activated Akt and COX-2 at the early stages of endometrial carcinoma development, with a corresponding decrease in miR199a and miR-101a, two microRNAs that inhibit COX-2 at the post transcription level. This would suggest the Pten-Akt-COX2 pathway is important in the initiation of tumor growth. In addition pharmacological inhibition of downstream Akt activation targets results in reduction of $Pten^{+/-}$ uterine lesion size (107).

Type I endometrial carcinomas are characterized by *Pten* mutations and are typically hormone responsive, the group ovariectomized $Pten^{pr-/-}/p53^{pr-/-}$ mutants at 3 weeks to investigate potential ovarian steroid regulation. Interestingly, ovariectomy had no detrimental impact on tumor formation rate when the mice were examined at 2 months of age suggesting, in this model, a limited role for ovarian steroid hormones in the progression of endometrial carcinomas. Other studies have found similar results with regards to the consequence of ovariectomizing $Pten^{+/-}$ mice and subsequent tumor development. Fyles *et al* (115), using $Pten^{+/-}$ mice on a C57BL/6 background generated from deletion of exon 5, showed that despite ovariectomy at 6 or 12 weeks, greater than 88% of mutant mice developed atypical endometrial hyperplasia or adenocarcinoma, comparable to 100% of control mice. Vilgelm *et al* (109), using $Pten^{+/-}$ on a C57BL/6 x 129/Sv background also found that ovarectomizing mutant mice at 3 weeks had no effect on the development of endometrial tumors, although they were reduced in size compared to $Pten^{+/+}$ controls. Again this would imply a lack of hormonal involvement in *Pten*-loss related endometrial carcinoma progression.

4.1.3. Kinases, kinase interacting proteins, inhibitors and endometrial cancer

The protein kinase family plays a key role in regulating the cell cycle, cell proliferation, survival, and DNA damage repair. Members of this family are amongst the most commonly mutated genes in cancer, and both mutated and activated protein kinases have proved to be tractable targets for anti-cancer therapies. In addition alterations in regulators of these kinases may result in malignant transformation of the cell.

4.1.3.1. Cables 1: cyclin-dependent kinase (cdk)-interacting protein

Cables-1 is encoded on chromosome 18q12 in humans. Cables-1 mediates an interaction between cdk2 and the non-receptor tyrosine kinase Wee-1 in proliferating cells. This interaction potentiates the inhibitory cdk tyrosine (Y15) phosphorylation by Wee-1 resulting in decreased cellular proliferation (116). Cables-1 is expressed at low levels in the estrogen dominant proliferative endometrium and at significantly higher levels in the progesterone dominant secretory endometrium. Treatment of endometrial cell lines with progesterone causes a dose-dependent increase in *cables-1* mRNA expression (117). This increase is negated by RU486 (mifepristone) suggesting that progesterone can regulate Cables 1 expression through the classic PR receptor mediated pathway. Its expression is significantly reduced, if not lost, in endometrial hyperplasia and in endometrial cancer (118) in humans. The *Cables 1* null female mice develop endometrial hyperplasia at a young age and have evidence of non-invasive carcinoma in response to chronic estrogen exposure which was not evident in their WT sisters (118).

Thus, this mouse model recapitulates that which is observed in humans and supports the fact that estrogen can serve as a catalyst in endometrial cancer. While Cables loss by itself contributes to a measurable phenotype there are more common models that display a more aggressive phenotype which may provide independent and/or synergistic signals promoting tumor progression.

4.1.3.2. LKB1: serine/threonine kinase

Another critical intracellular regulator of cell proliferation and cell survival is LKB1 (also known as STK11). This was the first serine/threonine kinase shown to have activity as a tumor suppressor (119). LKB1 directly activates AMP activated kinase, a sensor of cellular energy and AMP levels (120). $Lkb^{-/-}$ nullizygosity results in embryonic lethality by midgestation due to marked vascular defects (121). However, $Lkb^{-/+}$ mice exhibit a syndrome of gastrointestinal polyposis similar to that seen in Peutz-Jeghers syndrome in humans (122-125). Peutz-Jeghers syndrome in humans is characterized by hamartomatous polyps found throughout the gastrointestinal tract and mucocutaneous hyperpigmentation. Most individuals with Peutz-Jeghers syndrome have a mutation in LKB1 inherited in an autosomal dominant fashion (126, 127). Approximately 50% of people with Peutz-Jeghers Syndrome will develop epithelial malignancies by age 60 at a variety of anatomic sites (128, 129). Up to 13% of these are gynecologic cancers (ovarian, uterine or cervical) (130).

Interestingly, a recent paper by Contreras *et al* has shown that $Lkb^{-/+}$ mice develop endometrial adenocarcinomas at a high rate (122) (Figure 3). Of 15 females, 8 (53%) developed uterine tumors compared to 0 of 54 WT mice. These tumors were unusually well-differentiated, and at least 50% were large enough to be detectable on physical examination. The authors confirmed their findings by studying mice homozygous for a floxed *Lkb1* allele ($Lkb1^{L/L}$). Seventeen of these female mice were injected with Ad-Cre, and 11 (65%) developed uterine tumors compared to 0 of 30 WT mice. In both sets of mice, though well-differentiated, the tumors were characterized as being highly invasive. To confirm the relevance of these findings in humans, LKB1 expression was studied in an array of 190 human endometrial cancers. High grade tumors and tumors that were more deeply invasive in the myometrium were found to have lower LKB1 expression suggesting that LKB1 plays a role in the progression of human endometrial cancers similar to that seen in $Lkb1^{-/+}$ mice.

Though the mechanisms by which LKB1 mediates tumor suppression are not completely known, LKB1 does phosphorylate AMP-dependent kinase (AMPK) which in turn regulates mammalian target of rapamycin

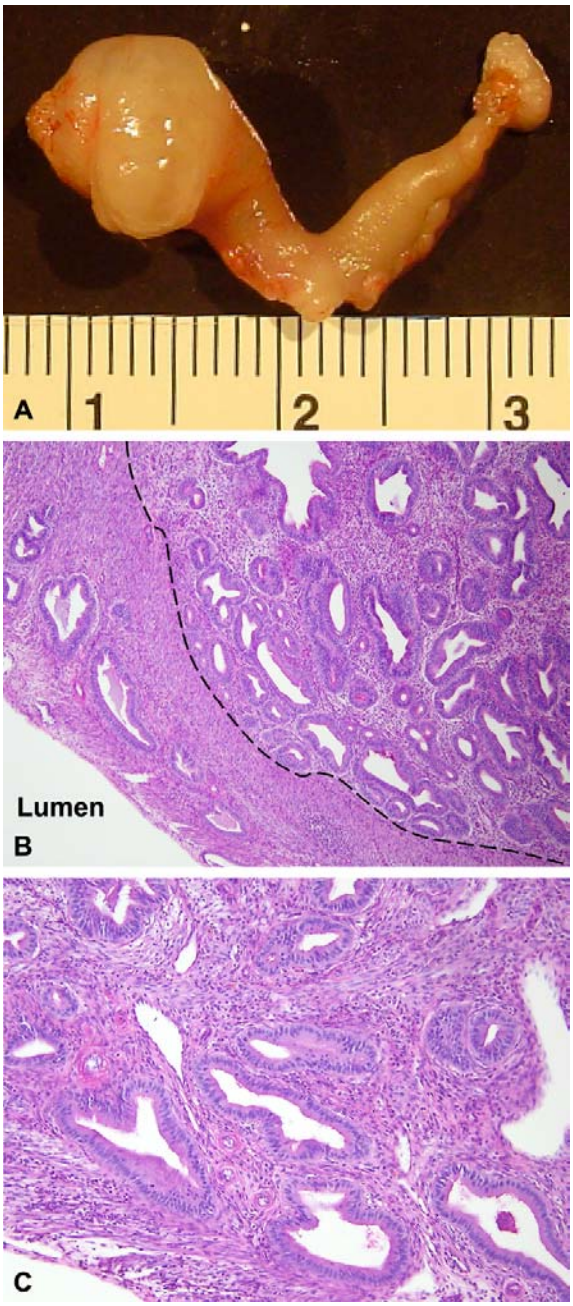

Figure 3. Uterus with tumor derived from the Lkb1 Ad Cre model (122). (A) Gross appearance of an invasive tumor confined to one uterine horn. (B) Microscopically, the tumor is composed of a proliferation of well-differentiated malignant glands that invade the myometrium. The dashed line demarcates the endometrial-myometrial interface. (C) Higher magnification of the LKB1 tumor showing myometrial invasion to the serosal surface of the uterus. The LKB1 model is comparable to a deeply invasive grade 1 endometrioid carcinoma in humans.

(mTOR) (131). LKB1 also, however, plays a role in the regulation of other kinases. It is likely the loss of LKB1 in controlling one or more of these pathways contribute to its role in tumor development.

4.1.3.3. Par4: kinase inhibition

The *Prostate apoptosis response 4* (*Par4; PAWR)* gene is located at chromosome 12q21 a region frequently found to have deletions in pancreatic and gastric cancers (132, 133). *Par4* was initially identified in a differential screen for genes that were up-regulated in a rat prostate cancer cell line in response to apoptotic stimuli. Further studies revealed that over-expression of *Par4* in human prostate and melanoma cell lines increased the cells response to these stimuli (134). Par4 is thought to act as an inhibitor of the atypical protein kinases (135) resulting in down regulation of pro-apoptotic effectors. Examination of primary renal cell carcinoma tissue reveal a significant loss (~64%) of Par4 expression in carcinoma samples when compared to control tissues (136).

Par4$^{-/-}$ mice, generated initially to investigate its role in cellular apoptosis, show increased tumor susceptibility to endometrial and prostate carcinomas in comparison to WT controls (135). Endometrial hyperplasia presented in ~80% of *Par4*$^{-/-}$ mice by 9 months, with 36% progressing to adenocarcinoma by 18-24 months in contrast to 0% of *Par4*$^{+/+}$ females. Western blot analyses of uteri protein lysates showed a significant increase in pro-apoptotic effector expression in *Par4*$^{-/-}$ uteri than in *Par4*$^{+/+}$ uteri. Interestingly, administration of estrogen (17β-estradiol) to *Par4*$^{+/+}$ mice decreased Par4 uterine levels further suggesting that decreased Par4 is associated with endometrial proliferation. Clinically Par4 has been described as a potential tumor suppressor (137) due to its decrease of expression (~40%) in primary type I endometrial carcinomas. No association was found between reduced Par4 expression and mutations of *Pten, k-ras or beta-catenin*. However, Par4 reduction was more frequent in ER positive tumors. Finally, hypermethlyation of the Par4 promoter was found in 32% of tumors that had reduced Par4 levels.

4.1.4. Replicative aging and endometrial abnormalities

Telomeres are a sequence of repetitive DNA located at the end of linear chromosomes where they serve to prevent the chromosome end from being recognized as DNA double-stranded breaks. Telomerase is the ribonucleoprotein reverse transcriptase complex that maintains telomere length and is reactivated in 90% of cancers (138). Telomere length does not always correlate to telomerase activity (139). Telomerase reverse transcriptase (TERT), part of the telomerase complex is generally not expressed in most differentiated somatic cells, therefore telomerase activity is absent. This absence leads to a progressive shortening of telomeres and cellular senescence. With the exception of the germline, telomere shortening thus occurs throughout the body as a function of increasing age, and this telomere attrition is believed to account for the striking increase in the incidence of most epithelial malignancies with advancing age (140). Cells that bypass senescence, acquire critically short telomeres resulting in chromosome instability, the result of which is implicated in the onset of some cancers (141).

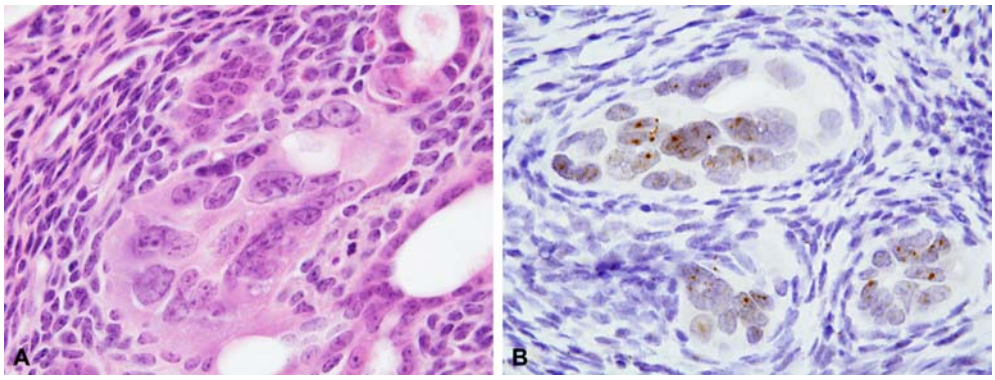

Figure 4. Photomicrographs of endometrial tumor derived from mTERC-/- G5i mice with short telomeres. (A) The carcinomas in these mice display extreme cytological and nuclear atypia, quite similar to that seen in human uterine serous carcinoma. (B) Immunohistochemistry for pH2AX, a marker of apoptosis. Note that these carcinomas have numerous pH2AX positive foci in the nuclei, consistent with genomic instability and double-strand DNA breaks due to critical telomere attrition.

4.1.4.1. m*TERC*

Because of this potential association between critically short telomeres and tumorigenesis, Akbay *et al* (142) generated m*TERC* deficient mice with shortened telomeres to investigate telomere attrition in the initiation and progression of endometrial cancer. Due to their short telomere length (in 50% of chromosomes) these mice (mTERC$^{-/-}$G5i) were prone to genomic instability. At 13-18 months no mTERC$^{-/-}$ G5i mouse had any evidence of endometrial adenocarcinoma. Eighty percent of mutants had pre-neoplastic endometrial pre-cancers which resembled endometrial intraepithelial carcinoma (EIC) (Figure 4). The glandular lesions have extreme cytological and nuclear atypical, comparable to uterine serous carcinoma in women. No invasive tumor growth or metastases were associated with these lesions. Control mice with stable telomere length, had no evidence of such lesions. Histological analyses for pH2AX, a marker of double stranded breaks, revealed multiple positive foci throughout the EIC lesions, indicating the presence of genomic instability. Regions of positive foci were also present in adjacent normal endometrium leading the authors to speculate that double stranded breaks may be a precursor for the formation of EIC-like lesions. No such staining was found in control or WT mice or in other tissues analysed, suggesting the endometrium may be more sensitive to double stranded breaks and genomic instability. Upon analyses of primary human type I and II endometrial cancers (See introduction to Section 4 for definition of type I vs. II endometrial cancer), the authors found decreased telomere lengths in both tumor types. Only type II tumors had shortened telomeres in adjacent normal epithelium leading the group to imply that telomere attrition may contribute to the progression of type I cancers and the initiation of type II cancers. These findings are consistent with the fact that *TP53* mutations are common in type II but not type I cancers, given that p53 is a well-established telomere checkpoint (143, 144) and also provide a possible explanation for the striking correlation with type II disease and advanced age.

5. UTERINE MESENCHYMAL TUMORS

Of all the uterine tumors, leiomyoma (a.k.a. fibroid) are the most prevalent and frequently diagnosed. They arise from the smooth muscle (myometrium) of the uterus. They express steroid receptors, are typically responsive to hormones, and decrease with the onset of menopause. There is also evidence to suggest that a genetic pre-disposition exists. Although benign they can have devastating results due to secondary complications (i.e. bleeding, pain, pressure, infertility and pregnancy related complications) (145). Leiomyomata can rarely undergo malignant transformation (146, 147). However, most leiomyosarcomas (malignant smooth muscle tumors) are believed to arise by distinct biological pathways and not from pre-existing leiomyomata.

Uterine sarcomas represent 4-9% of all uterine malignancies (148). Leiomyosarcoma (LMS) and endometrial stromal sarcoma (ESS) are the most common. Mouse models of both LMS and ESS have been created and utilized to improve understanding of these rare disorders.

5.1. Leiomyoma

5.1.1. Model presenting with leiomyoma

Teixeira and colleagues (149) have utilized the Cre recombinase activated MISIIR promoter locus to constitutively activate beta-catenin in the uterine mesenchyme. The females presented with evidence of myometrial hyperplasia similar to that described by others (54). However, in this model the mice developed mesenchymal tumors with a 100% penetrance rate (Figure 5). Interestingly, the tumors exhibited histological and molecular characteristics typically seen of human leiomyomata (i.e. increased TGFbeta and mammalian target of mTOR). This is especially important since there are only a limited number of *in vivo* models. The most heavily relied upon *in vivo* model is the Eker rat which has a tuberous sclerosis 2 mutation resulting in uterine smooth muscle tumor development in aged animals (150). The development of a mouse model that presents with hormonally responsive tumors resembling leiomyoma opens up a wealth of possibilities ranging from further development of the model to preclinical testing of novel therapeutics.

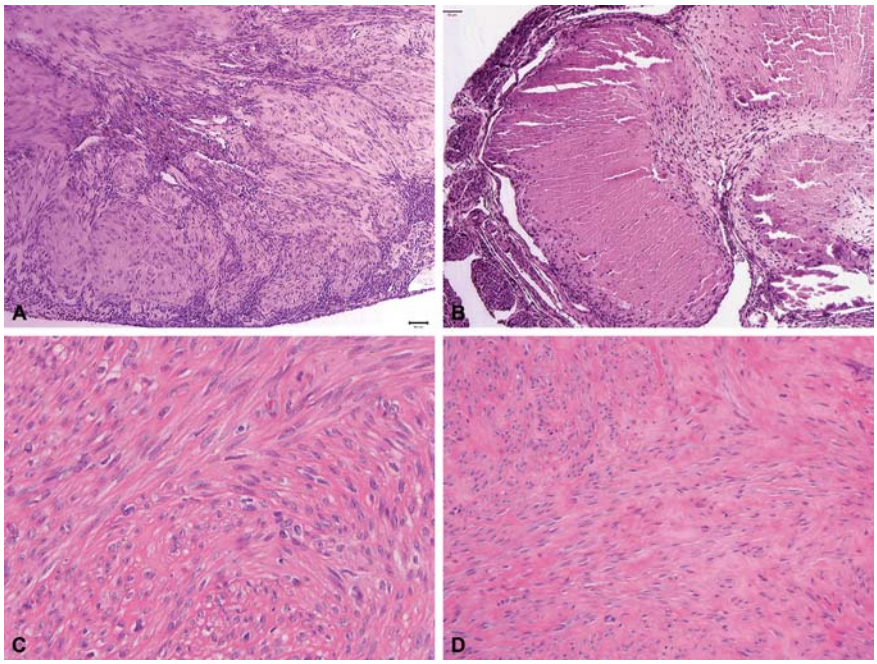

Figure 5. Photomicrographs of mouse (A and B) and human (C and D) uterine leiomyoma. Leiomyomata are very common smooth muscle tumors of the uterus. Despite their benign nature, they are clinically significant causes of dysfunctional uterine bleeding, pelvic pain, and infertility. (A) Constitutively activated beta-catenin in the uterine mesenchyme results in female mice with myometrial hyperplasia. In this particular model developed by Teixeira and colleagues (149) the mice develop mesenchymal tumors, including leiomyomas, with a 100% penetrance rate. (B) In this mouse model, as the leiomyomas get larger, they become extensively sclerotic. (C) Human uterine leiomyoma is composed of a proliferation of spindle-shaped smooth muscle cells. (D) Human leiomyomata are commonly associated with the deposition of collagen and extracellular matrix (eosinophilic material between tumor cells), similar to the mouse model.

5.2. Leiomyosarcoma

5.2.1. Models presenting with leiomyosarcoma

5.2.1.1. CaBP9k/Tag

One of the first mouse models of uterine LMS utilized over-expression of a simian virus early region (SVER) expressing the 40 T antigen (Tag) oncoproteins driven by the promoter of the Calbindin-D9K (CaBP9K) gene (151). These over-expressing mice developed LMS throughout the female reproductive tract, though primarily within the uterus (90%).

5.2.1.2. mogp-Tag

Given this association between the simian virus Tag and LMS, others utilized a 5'-flanking sequence of the mouse oviduct-specific glycoprotein (OGP) gene to drive SVER expression, confirming the female reproductive tract genesis of LMS (123). This group then ovariectomized these over-expressing transgenic mice (mogp-Tag), and demonstrated that tumors would form only under the influence of estrogen, clearly suggesting that tumorigenesis was estrogen dependent.

5.2.1.3. Beta-actin $^{SVER/+}$/HS-cre1

More recently, Politi and colleagues have refined over-expression of simian virus 40 Tag oncoproteins utilizing a *cre/loxP* recombination process (152). These investigators developed "*Hs-cre*" transgenic mice that over-expressed *cre* and crossed these animals with dormant SV 40 Tag oncoproteins with *loxP* sites upstream leading to ubiquitous oncoprotein expression when spliced (beta-actin $^{SVER/+}$/HS-*cre1)*. In a recent study, these authors demonstrated a near complete phenotypic penetrance with a uniform transformation of myometrium to LMS (152).

5.2.1.4. MMTV-CR-1

Investigations with the epidermal growth factor family Cripto-1(CR-1) protein have demonstrated that 20% of FVB/N mice (MMTV-CR-1) that over-express this protein develop LMS (153). This study demonstrated that none of the control mice developed uterine tumors, and the tumors that did form expressed high levels of beta-catenin, P-src, P-Akt and P-GSK utilizing western blot and immunohistochemistry. These data suggest CR-1 to be a mediator of sarcomatous tumorigenesis via specific pathways (153). Both the CR-1 and SVER models provide models that can generate LMS in mice with a high frequency leading to future investigations to better understand the specific mechanisms that these pathways utilize.

5.2.1.5. LMP2

Intracellular antigens are presented to cytotoxic T lymphocytes through the major histocompatibility complex (MHC) class I molecule. Low molecular mass polypeptide-2 (LMP2) is a proteasome subunit involved in peptide generation and presentation to the MHC class I molecule.

Defects in the MHC class I antigen-processing machinery, including LMP2, have been described in various human tumors including melanoma, breast, cervical, renal and small cell lung (reviewed in (154)). $LMP2^{-/-}$ mice have impaired proteasome function (155, 156) and are prone to development of uterine neoplasms (157). Hayashi and Faustman (157), reported 10/28 $LMP2^{-/-}$ mice having LMS by 12 months of age in comparison to age matched controls. Additionally, LMS of the mutant mice showed high levels of Ki-67 staining. No human LMS samples were analyzed in this study. However, reduced expression at the protein level was recently documented in human LMS (158).

5.2.1.6. E2F-1

E2F-1, a transcription factor from the E2F family, plays a role in cell cycle regulation and cell proliferation as well as being involved in tumor suppressor protein function, specifically the retinoblastoma tumor suppressor protein (pRB). Yamakasi *et al* (159) assessed the role of E2F in normal growth and development and whether E2F-1 has a critical function in the regulation of proliferation, apoptosis, and/or differentiation *in vivo*. To accomplish this, the E2F-1 locus in mice was inactivated by homologous recombination. This group found that loss of E2F-1 predisposed many of the homozygous $E2F\text{-}1^{-/-}$ mutants to develop tumors, including uterine tumors.

In 8/20 of homozygous $E2F\text{-}1^{-/-}$ mutants an aggressive sarcoma developed within the uterine horns. The mean time to development of the tumors was 13.7 months. Histologically these sarcomas appeared to be either an endometrial stromal sarcoma or a histiocytic sarcoma. These tumors also appeared to have a very high metastatic potential. Six of the 11 reproductive tract sarcomas (both male and female) demonstrated numerous metastatic sites throughout the abdomen. The argument was made that E2F-1 has a key growth suppressive role in the uterine horns of females. However, the incidence and long latency for the development of these sarcomas support the involvement of additional mutations.

5.3. Endometrial stromal sarcoma

5.3.1. Models presenting with lesions resembling endometrial stromal sarcoma

Mouse models with heterozygous mutant p53 genes ($p53^{val135/wt}$) have been extensively utilized to develop *in vivo* ESS, clearly demonstrating the link between p53 and the pathogenesis of uterine sarcomas. Two major backgrounds have been used, WT and heterozygous *p53* CBA and C57BL/6J crossed with the UL53-3 mutants to generate heterozygous *p53*-deficient mice. Both lines of investigation treated *p53* WT and deficient mice with mutagenic substances and reliably derived ESS.

5.3.1.1. $p53^{val135/w}$

Using UL53-3 transgenic p53 mice with a Ala135Val germ-line mutation crossed with C57BL/6J mice, Zhang and colleagues treated both $p53^{wt/wt}$ and $p53^{val135/w}$animals with 1,2 dimethylhydrazine, a known carcinogen that can induce colon carcinomas (160). These authors demonstrated that in addition to colon carcinomas, 90% of the $p53^{+/-}$ animals developed ESS compared to 10% of the $p53^{wt/wt}$. Further investigation with RT-PCR and northern blot of common p53 associated targets demonstrated that the Reprimo gene was highly expressed in WT uteri, with nearly complete inhibition in the sarcomas suggesting both lack of p53 and decreased Reprimo activity to be key factors in promoting the generation of ESS (160).

5.3.1.2. $p53^{+/-}$ CBA

Extensive studies evaluating ESS have also been conducted utilizing *p53* WT and deficient CBA mice after the administration of N-ethyl-N-nitrosurea (ENU), a known endocrine disrupting chemical that can induce carcinogenesis (161). In this study, 94% of p53 mutants developed ESS compared to 37% in the WT cohort leading the authors to conclude that this model could be useful for both functional and chemo-preventive studies of this rare tumor (161). This group further utilized their ESS model to determine whether tumorigenesis was hormonally modulated by creating a four armed experimental design (162). All arms underwent pretreatment with ENU, and then received one of 4 regimens: [1] no further treatment, [2] diet with 1 ppm ethinylestradiol (EE_2) [3] diet with 5 ppm EE_2 x 4 weeks, then 2.5 ppm EE_2 [4]. Diet with 2000 ppm Methoxychlor, a known estrogenic pesticide. They demonstrated that 87% of the *p53* mutants in group 3 that received the most EE_2 developed ESS compared to 47% of those mutants in group 1 that received no further treatment. These data suggest not only that p53 is an important factor in the development of ESS, but also that estrogen can promote the growth of this class of uterine sarcoma (162).

6. OTHER UTERINE TUMORS

6.1. MMTV betaTcrp1

Beta-transduction repeat-containing protein 1 (betaTrcp1) is a component of the F-box protein complex that regulates cellular levels of various proteins by functioning as a protein ligase that degrades beta-catenin, IkappaB (inhibitor of nuclear factor kappa B [NF-kappaB]) (163) and Emi1 (cell cycle regulator protein)(164). Evidence also leads to betaTrcp1 being a regulator of the cell cycle (165). The gene encoding betaTrcp1 has been mapped to chromosome 10q24, a region associated with deletions in prostate (166) and breast cancers (167). Kudo *et al* (168) in their investigation of mammary gland development generated transgenic mice expressing *betaTrcp1* targeted specifically to epithelial cells using a MMTV promoter. MMTV *betaTrcp1* mice developed mammary hyperplasia (80%) adenocarcinoma (4%), ovarian (8%) and uterine (3%) tumors. Although the percentage of mice generating tumors was small, the total number of mice that developed tumors was still significantly greater than their WT counterparts. The authors documented $betaTrcp1^{-/-}$ (not under MMTV control) mice as having defective mammary gland development which corresponded to a decrease in cell proliferation as measured by BrdU incorporation. Kudo

(168) investigated various proteins involved in cell cycle regulation in mammary glands from both transgenic and WT mice. However no similar results were reported for uterine tissues as the primary focus of this study was the investigation of mammary development and tumorigenesis.

7. UTILITY OF THE MOUSE MODELS IN STUDYING HUMAN DISEASE

The value of mouse models in illustrating cause and effect relationships is obvious. However the utility or practicality of the individual models often becomes an issue. When evaluating the contributions of the individual mutant mouse models we have to take into account their individual penetrance rate. If penetrance rate is low it is often difficult to determine whether the onset of the tumors is more in favor of chance. Secondly, the tissue specificity is important. In a number of models presented herein there was evidence of more than one tumor type. More often than not uterine tumors develop later in life and are much slower growing, suggesting that the mice may succumb to complications related to non-uterine tumors before realizing the value of the model to the development of progression of uterine tumors. Albeit the models remain useful, their overall value is lessened.

Of all the mouse models that have any application to human disease, the PTEN mutant is the best recognized and most extensively studied. This is in direct contrast to those lesser-known models whereby genetic mutations resulted in uterine anomalies. Of these there is limited clinical supportive data, if any at all, to suggest that they may have direct relevance to the human uterine disease. However, it may also be that tumors derived from these types of mutations are so infrequent that their impact is diluted in the general population. Interestingly, while the incidence of leiomyoma is so prevalent there are few mouse models available.

Similarly, while endometrial cancer is the most prevalent gynecological malignancy there are only a few models that show evidence of highly invasive tumors. There are however, a number of mouse models that have evidence of hyperplasia and low-grade malignancies which in the future can be crossed to determine the effects of multiple hits. This has proven useful in mouse models of ovarian cancer (169, 170). It will also be important to generate more mouse models that develop non-endometrioid carcinomas to aid in our understanding of the more spontaneous tumors often linked to a poor outcome.

8. CURRENT AND PROPOSED CRITERIA FOR DESCRIBING TUMOR PATHOLOGY IN MUTANT MOUSE MODELS

Although we advocate that uterine pathologies in mouse models be reviewed by an experienced pathologist, it must be emphasized that some caution be exercised when comparing mouse pathology to human pathology. Certain microscopic criteria developed to distinguish various human pathologies may be difficult to apply in mice. In reviewing mouse models of endometrial hyperplasia and carcinoma, we found it difficult to reliably distinguish endometrial hyperplasia from endometrial carcinoma. The presence of neoplastic glands within the myometrium, however, is a definite indicator of invasion, and hence endometrial carcinoma. At the present time, we propose that the presence of definitive myometrial invasion (defined as the presence of tumor glands within the myometrial layer), at least in some animals, be considered a requirement for a model to be considered a model of invasive endometrial carcinoma. While we recognize that some *bona fide* human endometrial carcinomas do not invade the myometrial layer, it is also the case that the distinction between endometrial hyperplasia and carcinoma can be quite difficult in human pathological samples. Although histologic criteria exist for the distinction of hyperplasia and carcinoma (e.g., lack of intervening stroma) there is in fact a continuum of histological changes making it difficult, at least in some cases, to make a definitive assignment.

Along these lines, the diagnosis of endometrial hyperplasia in the mouse is complicated by a variety of factors including cyclical variation in endometrial histology and age-related changes in the endometrium. Aging female mice tend to accumulate fluid-filled cysts in the endometrium that should not be confused with true hyperplasia. Further complicating the diagnosis of hyperplasia are the cyclical histological changes that characterize endometrial histology. Although not as prominent as those which occur in women, these changes may nonetheless obscure interpretation of endometrial lesions in the mouse. Possible criteria for hyperplasia thus include 1) differences in overall uterine weight (following puncture to remove excess fluid) and 2) hypercellularity evident as cribriforming or other abnormal architectural patterns diagnostic of hyperplasia. For the latter, consideration should be given to performing the analyses on estrus-dated animals or in a sufficiently large number of animals (e.g. n=10) to reduce the possibility that variations in cycling account for any observed histologic phenotypes.

9. PERSPECTIVES

Endometrial cancer is the most common gynecologic cancer and its incidence is likely to further increase due to the continued rise in societal obesity, a major risk factor. From a mouse modeling perspective we have to be open to considering genetic-environmental alterations (i.e., obesity) that may ultimately result in uterine tumors. There are a number of mouse models that display obese phenotypes, however to our knowledge none of these mouse models have been critically evaluated for evidence of endometrial hyperplasia and/or cancer. Alternatively, mouse models which display diabetic or hypertensive symptoms (171-175), both of which are associated with increased risk of uterine cancer may also serve to shed some light in the malignant transformation of the uterine tissue.

Complex atypical hyperplasia and type I endometrial cancer will continue to be primary areas of

interest. While low-stage tumors can be cured with surgery, there is a great need for a better understanding of the molecular steps driving endometrial progression, which will facilitate the development of targeted therapies against advanced tumors. Mouse models will also become increasingly important with the movement towards personalized therapy. It is anticipated that the delineation of many of the contributing signaling pathways will be either tested or confirmed via genetically manipulated mouse models.

Standard chemotherapy is largely ineffective for any type of metastatic disease, including type II endometrial tumors. Development of mouse models that display characteristics of type II endometrial cancer would be very helpful in defining the underlying mechanisms driving the malignant transformation in the endometrial serous, mucinous and clear cell cancers.

Preclinical testing can be accomplished in molecular identified targets in the more common endometrial cancers. Additionally, mouse models may be even more important for rare tumors (LMS, ESS) if causative mutations can be discovered (i.e., FOXL2 in granulosa cell ovarian tumors (176), EGFR in squamous cell carcinoma of the vulva (177)). These types of studies do not have to be limited to tumors associated with the endometrium. Fibroids are exceedingly common and the number one cause of hysterectomy and fertility loss. Mouse models of leiomyoma could serve to test targeted agents that can inhibit signaling pathways attributed to aberrant growth factor signaling, disruption of apoptosis or dysregulated wound healing. Similarly to the rare tumors of the endometrium, the number of mutant mouse models presenting with phenotypes of uterine sarcoma would suggest that we may have already uncovered some potential targets for slowing the progression of the disease or at the minimum may be contributing to the pathology of the disease.

A number of mouse models of uterine tumors have been generated which faithfully recapitulate many aspects of the human diseases. The challenge upon us is to maximize the utility of these current models, to further refine them, and to exploit them in preclinical and other types of studies to improve diagnostic and therapeutic options in the clinic.

10. ACKNOWLEDGEMENTS

The preparation of this review was funded in part by Advanced Medical Research Foundation, Vincent Obstetrics and Gynecology Service Research Funds, NIH CA98333, and Uterine Cancer SPORE NIH 1P50CA098258-01. We would also like to recognize Dr. Rosemary Foster for her careful review.

11. REFERENCES

1. E. Sivridis and A. Giatromanolaki: The endometrial hyperplasias revisited. *Virchows Arch*, 453(3), 223-31 (2008)

2. J. M. Spicer, I. Siebert and T. F. Kruger: Postmenopausal bleeding: a diagnostic approach for both private and public sectors. *Gynecol Obstet Invest*, 61(3), 174-8 (2006)

3. J. K. Panda: One-stop clinic for postmenopausal bleeding. *J Reprod Med*, 47(9), 761-6 (2002)

4. F. Linkov, R. Edwards, J. Balk, Z. Yurkovetsky, B. Stadterman, A. Lokshin and E. Taioli: Endometrial hyperplasia, endometrial cancer and prevention: gaps in existing research of modifiable risk factors. *Eur J Cancer*, 44(12), 1632-44 (2008)

5. M. Epplein, S. D. Reed, L. F. Voigt, K. M. Newton, V. L. Holt and N. S. Weiss: Risk of complex and atypical endometrial hyperplasia in relation to anthropometric measures and reproductive history. *Am J Epidemiol*, 168(6), 563-70; discussion 571-6 (2008)

6. A. T. Hertig, S. C. Sommers and H. Bengloff: Genesis of endometrial carcinoma; carcinoma *in situ*. *Cancer*, 2(6), 964-71, illust (1949)

7. F. Vellios: Endometrial hyperplasia and carcinoma *in situ*. *Gynecol Oncol*, 2(2-3), 152-61 (1974)

8. W. B. Wentz: Progestin therapy in endometrial hyperplasia. *Gynecol Oncol*, 2(2-3), 362-7 (1974)

9. H. K. Beutler, M. B. Dockerty and L. M. Randall: Precancerous lesions of the endometrium. *Am J Obstet Gynecol*, 86, 433-43 (1963)

10. R. E. Scully: Definition of precursors in gynecologic cancer. *Cancer*, 48(2 Suppl), 531-7 (1981)

11. R. J. Kurman, P. F. Kaminski and H. J. Norris: The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. *Cancer*, 56(2), 403-12 (1985)

12. J. L. Hecht and G. L. Mutter: Molecular and pathologic aspects of endometrial carcinogenesis. *J Clin Oncol*, 24(29), 4783-91 (2006)

13. C. L. Trimble, J. Kauderer, R. Zaino, S. Silverberg, P. C. Lim, J. J. Burke, 2nd, D. Alberts and J. Curtin: Concurrent endometrial carcinoma in women with a biopsy diagnosis of atypical endometrial hyperplasia: a Gynecologic Oncology Group study. *Cancer*, 106(4), 812-9 (2006)

14. M. E. Sherman: Theories of endometrial carcinogenesis: a multidisciplinary approach. *Mod Pathol*, 13(3), 295-308 (2000)

15. K. Ito, H. Utsunomiya, N. Yaegashi and H. Sasano: Biological roles of estrogen and progesterone in human endometrial carcinoma--new developments in potential endocrine therapy for endometrial cancer. *Endocr J*, 54(5), 667-79 (2007)

16. P. Kastner, A. Krust, B. Turcotte, U. Stropp, L. Tora, H. Gronemeyer and P. Chambon: Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *Embo J*, 9(5), 1603-14 (1990)

17. U. Sartorius, I. Schmitz and P. H. Krammer: Molecular mechanisms of death-receptor-mediated apoptosis. *Chembiochem*, 2(1), 20-9 (2001)

18. W. T. Schrader and B. W. O'Malley: Progesterone-binding components of chick oviduct. IV. Characterization of purified subunits. *J Biol Chem*, 247(1), 51-9 (1972)

19. O. M. Conneely, B. L. Maxwell, D. O. Toft, W. T. Schrader and B. W. O'Malley: The A and B forms of the chicken progesterone receptor arise by alternate initiation of translation of a unique mRNA. *Biochem Biophys Res Commun*, 149(2), 493-501 (1987)

20. W. Kleine, A. Fuchs and A. Pfleiderer: The importance of gestagen, tamoxifen and steroid receptors in the therapy of endometrial cancer. Report of an experiment. *Acta Obstet Gynecol Scand*, 61(5), 449-53 (1982)

21. R. Mortel, R. J. Zaino and P. G. Satyaswaroop: Designing a schedule of progestin administration in the control of endometrial carcinoma growth in the nude mouse model. *Am J Obstet Gynecol*, 162(4), 928-34; discussion 934-6 (1990)

22. E. E. Baulieu: Contragestion with RU 486: a new approach to postovulatory fertility control. *Acta Obstet Gynecol Scand Suppl*, 149, 5-8 (1989)

23. C. L. Clarke and R. L. Sutherland: Progestin regulation of cellular proliferation. *Endocr Rev*, 11(2), 266-301 (1990)

24. J. P. Lydon, F. J. DeMayo, C. R. Funk, S. K. Mani, A. R. Hughes, C. A. Montgomery, Jr., G. Shyamala, O. M. Conneely and B. W. O'Malley: Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev*, 9(18), 2266-78 (1995)

25. B. Mulac-Jericevic, R. A. Mullinax, F. J. DeMayo, J. P. Lydon and O. M. Conneely: Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. *Science*, 289(5485), 1751-4 (2000)

26. S. M. El-Naggar, M. T. Malik, A. Martin, J. P. Moore, M. Proctor, T. Hamid and S. S. Kakar: Development of cystic glandular hyperplasia of the endometrium in Mullerian inhibitory substance type II receptor-pituitary tumor transforming gene transgenic mice. *J Endocrinol*, 194(1), 179-91 (2007)

27. A. W. Burgess: EGFR family: structure physiology signalling and therapeutic targets. *Growth Factors*, 26(5), 263-74 (2008)

28. N. V. Marozkina, S. M. Stiefel, H. F. Frierson, Jr. and S. J. Parsons: MMTV-EGF receptor transgene promotes preneoplastic conversion of multiple steroid hormone-responsive tissues. *J Cell Biochem*, 103(6), 2010-8 (2008)

29. R. Brandt, R. Eisenbrandt, F. Leenders, W. Zschiesche, B. Binas, C. Juergensen and F. Theuring: Mammary gland specific hEGF receptor transgene expression induces neoplasia and inhibits differentiation. *Oncogene*, 19(17), 2129-37 (2000)

30. I. Ferby, M. Reschke, O. Kudlacek, P. Knyazev, G. Pante, K. Amann, W. Sommergruber, N. Kraut, A. Ullrich, R. Fassler and R. Klein: Mig6 is a negative regulator of EGF receptor-mediated skin morphogenesis and tumor formation. *Nat Med*, 12(5), 568-73 (2006)

31. K. L. Lee, K. R. Isham, L. Stringfellow, R. Rothrock and F. T. Kenney: Molecular cloning of cDNAs cognate to genes sensitive to hormonal control in rat liver. *J Biol Chem*, 260(30), 16433-8 (1985)

32. A. Makkinje, D. A. Quinn, A. Chen, C. L. Cadilla, T. Force, J. V. Bonventre and J. M. Kyriakis: Gene 33/Mig-6, a transcriptionally inducible adapter protein that binds GTP-Cdc42 and activates SAPK/JNK. A potential marker transcript for chronic pathologic conditions, such as diabetic nephropathy. Possible role in the response to persistent stress. *J Biol Chem*, 275(23), 17838-47 (2000)

33. P. O. Hackel, M. Gishizky and A. Ullrich: Mig-6 is a negative regulator of the epidermal growth factor receptor signal. *Biol Chem*, 382(12), 1649-62 (2001)

34. D. Fang, T. K. Nguyen, K. Leishear, R. Finko, A. N. Kulp, S. Hotz, P. A. Van Belle, X. Xu, D. E. Elder and M. Herlyn: A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res*, 65(20), 9328-37 (2005)

35. L. Fiorentino, C. Pertica, M. Fiorini, C. Talora, M. Crescenzi, L. Castellani, S. Alema, P. Benedetti and O. Segatto: Inhibition of ErbB-2 mitogenic and transforming activity by RALT, a mitogen-induced signal transducer which binds to the ErbB-2 kinase domain. *Mol Cell Biol*, 20(20), 7735-50 (2000)

36. C. Li, D. G. Heidt, P. Dalerba, C. F. Burant, L. Zhang, V. Adsay, M. Wicha, M. F. Clarke and D. M. Simeone: Identification of pancreatic cancer stem cells. *Cancer Res*, 67(3), 1030-7 (2007)

37. N. Jin, J. L. Gilbert, R. R. Broaddus, F. J. Demayo and J. W. Jeong: Generation of a Mig-6 conditional null allele. *Genesis*, 45(11), 716-21 (2007)

38. S. Thakur, H. B. Zhang, Y. Peng, H. Le, B. Carroll, T. Ward, J. Yao, L. M. Farid, F. J. Couch, R. B. Wilson and B. L. Weber: Localization of BRCA1 and a splice variant identifies the nuclear localization signal. *Mol Cell Biol*, 17(1), 444-52 (1997)

39. C. A. Wilson, M. N. Payton, G. S. Elliott, F. W. Buaas, E. E. Cajulis, D. Grosshans, L. Ramos, D. M. Reese, D. J.

Slamon and F. J. Calzone: Differential subcellular localization, expression and biological toxicity of BRCA1 and the splice variant BRCA1-delta11b. *Oncogene*, 14(1), 1-16 (1997)

40. D. Niederacher, H. X. An, S. Camrath, S. I. Dominik, U. J. Gohring, A. Oertel, M. Grass, P. Hantschmann, M. R. Lordnejad and M. W. Beckmann: Loss of heterozygosity of BRCA1, TP53 and TCRD markers analysed in sporadic endometrial cancer. *Eur J Cancer*, 34(11), 1770-6 (1998)

41. D. A. Levine, O. Lin, R. R. Barakat, M. E. Robson, D. McDermott, L. Cohen, J. Satagopan, K. Offit and J. Boyd: Risk of endometrial carcinoma associated with BRCA mutation. *Gynecol Oncol*, 80(3), 395-8 (2001)

42. M. E. Beiner, A. Finch, B. Rosen, J. Lubinski, P. Moller, P. Ghadirian, H. T. Lynch, E. Friedman, P. Sun and S. A. Narod: The risk of endometrial cancer in women with BRCA1 and BRCA2 mutations. A prospective study. *Gynecol Oncol*, 104(1), 7-10 (2007)

43. S. S. Kim, L. Cao, S. C. Lim, C. Li, R. H. Wang, X. Xu, R. Bachelier and C. X. Deng: Hyperplasia and spontaneous tumor development in the gynecologic system in mice lacking the BRCA1-Delta11 isoform. *Mol Cell Biol*, 26(18), 6983-92 (2006)

44. D. M. Bortner and M. P. Rosenberg: Induction of mammary gland hyperplasia and carcinomas in transgenic mice expressing human cyclin E. *Mol Cell Biol*, 17(1), 453-9 (1997)

45. S. S. Kim, L. Cao, C. Li, X. Xu, L. J. Huber, L. A. Chodosh and C. X. Deng: Uterus hyperplasia and increased carcinogen-induced tumorigenesis in mice carrying a targeted mutation of the Chk2 phosphorylation site in Brca1. *Mol Cell Biol*, 24(21), 9498-507 (2004)

46. A. Sullivan, M. Yuille, C. Repellin, A. Reddy, O. Reelfs, A. Bell, B. Dunne, B. A. Gusterson, P. Osin, P. J. Farrell, I. Yulug, A. Evans, T. Ozcelik, M. Gasco and T. Crook: Concomitant inactivation of p53 and Chk2 in breast cancer. *Oncogene*, 21(9), 1316-24 (2002)

47. D. W. Bell, J. M. Varley, T. E. Szydlo, D. H. Kang, D. C. Wahrer, K. E. Shannon, M. Lubratovich, S. J. Verselis, K. J. Isselbacher, J. F. Fraumeni, J. M. Birch, F. P. Li, J. E. Garber and D. A. Haber: Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science*, 286(5449), 2528-31 (1999)

48. N. Haruki, H. Saito, Y. Tatematsu, H. Konishi, T. Harano, A. Masuda, H. Osada, Y. Fujii and T. Takahashi: Histological type-selective, tumor-predominant expression of a novel CHK1 isoform and infrequent *in vivo* somatic CHK2 mutation in small cell lung cancer. *Cancer Res*, 60(17), 4689-92 (2000)

49. A. Reddy, M. Yuille, A. Sullivan, C. Repellin, A. Bell, J. A. Tidy, D. J. Evans, P. J. Farrell, B. Gusterson, M. Gasco and T. Crook: Analysis of CHK2 in vulval neoplasia. *Br J Cancer*, 86(5), 756-60 (2002)

50. S. Okada and T. Ouchi: Cell cycle differences in DNA damage-induced BRCA1 phosphorylation affect its subcellular localization. *J Biol Chem*, 278(3), 2015-20 (2003)

51. O. A. Mohamed, M. Jonnaert, C. Labelle-Dumais, K. Kuroda, H. J. Clarke and D. Dufort: Uterine Wnt/beta-catenin signaling is required for implantation. *Proc Natl Acad Sci U S A*, 102(24), 8579-84 (2005)

52. P. Machin, L. Catasus, C. Pons, J. Munoz, X. Matias-Guiu and J. Prat: CTNNB1 mutations and beta-catenin expression in endometrial carcinomas. *Hum Pathol*, 33(2), 206-12 (2002)

53. M. Saegusa, M. Hashimura, T. Yoshida and I. Okayasu: beta- Catenin mutations and aberrant nuclear expression during endometrial tumorigenesis. *Br J Cancer*, 84(2), 209-17 (2001)

54. J. W. Jeong, H. S. Lee, H. L. Franco, R. R. Broaddus, M. M. Taketo, S. Y. Tsai, J. P. Lydon and F. J. DeMayo: beta-catenin mediates glandular formation and dysregulation of beta-catenin induces hyperplasia formation in the murine uterus. *Oncogene*, 28(1), 31-40 (2009)

55. M. Saegusa, M. Hashimura, T. Kuwata, M. Hamano and I. Okayasu: Beta-catenin simultaneously induces activation of the p53-p21WAF1 pathway and overexpression of cyclin D1 during squamous differentiation of endometrial carcinoma cells. *Am J Pathol*, 164(5), 1739-49 (2004)

56. M. Saegusa and I. Okayasu: Frequent nuclear beta-catenin accumulation and associated mutations in endometrioid-type endometrial and ovarian carcinomas with squamous differentiation. *J Pathol*, 194(1), 59-67 (2001)

57. S. Kazerounian, K. O. Yee and J. Lawler: Thrombospondins in cancer. *Cell Mol Life Sci*, 65(5), 700-12 (2008)

58. K. Obata, S. J. Morland, R. H. Watson, A. Hitchcock, G. Chenevix-Trench, E. J. Thomas and I. G. Campbell: Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors. *Cancer Res*, 58(10), 2095-7 (1998)

59. G. Matarese, C. Alviggi, V. Sanna, J. K. Howard, G. M. Lord, C. Carravetta, S. Fontana, R. I. Lechler, S. R. Bloom and G. De Placido: Increased leptin levels in serum and peritoneal fluid of patients with pelvic endometriosis. *J Clin Endocrinol Metab*, 85(7), 2483-7 (2000)

60. M. Yamaguchi, K. Sugio, K. Ondo, T. Yano and K. Sugimachi: Reduced expression of thrombospondin-1

correlates with a poor prognosis in patients with non-small cell lung cancer. *Lung Cancer*, 36(2), 143-50 (2002)

61. T. Wakiyama, T. Shinohara, T. Shirakusa, A. S. John and G. P. Tuszynski: The localization of thrombospondin-1 (TSP-1), cysteine-serine-valine-threonine-cysteine-glycine (CSVTCG) TSP receptor, and matrix metalloproteinase-9 (MMP-9) in colorectal cancer. *Histol Histopathol*, 16(2), 345-51 (2001)

62. A. Ludlow, K. O. Yee, R. Lipman, R. Bronson, P. Weinreb, X. Huang, D. Sheppard and J. Lawler: Characterization of integrin beta6 and thrombospondin-1 double-null mice. *J Cell Mol Med*, 9(2), 421-37 (2005)

63. S. Zhang, C. Balch, M. W. Chan, H. C. Lai, D. Matei, J. M. Schilder, P. S. Yan, T. H. Huang and K. P. Nephew: Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res*, 68(11), 4311-20 (2008)

64. A. N. Elayadi, K. N. Samli, L. Prudkin, Y. H. Liu, A. Bian, X. J. Xie, Wistuba, II, J. A. Roth, M. J. McGuire and K. C. Brown: A peptide selected by biopanning identifies the integrin alphavbeta6 as a prognostic biomarker for nonsmall cell lung cancer. *Cancer Res*, 67(12), 5889-95 (2007)

65. N. Ahmed, C. Riley, G. E. Rice, M. A. Quinn and M. S. Baker: Alpha(v)beta(6) integrin-A marker for the malignant potential of epithelial ovarian cancer. *J Histochem Cytochem*, 50(10), 1371-80 (2002)

66. M. A. Behera, L. Feng, B. Yonish, W. Catherino, S. H. Jung and P. Leppert: Thrombospondin-1 and thrombospondin-2 mRNA and TSP-1 and TSP-2 protein expression in uterine fibroids and correlation to the genes COL1A1 and COL3A1 and to the collagen cross-link hydroxyproline. *Reprod Sci*, 14(8 Suppl), 63-76 (2007)

67. J. L. Hecht, B. M. Dolinski, H. A. Gardner, S. M. Violette and P. H. Weinreb: Overexpression of the alphavbeta6 integrin in endometrial cancer. *Appl Immunohistochem Mol Morphol*, 16(6), 543-7 (2008)

68. S. Hazelbag, G. G. Kenter, A. Gorter, E. J. Dreef, L. A. Koopman, S. M. Violette, P. H. Weinreb and G. J. Fleuren: Overexpression of the alpha v beta 6 integrin in cervical squamous cell carcinoma is a prognostic factor for decreased survival. *J Pathol*, 212(3), 316-24 (2007)

69. L. A. Van Aarsen, D. R. Leone, S. Ho, B. M. Dolinski, P. E. McCoon, D. J. LePage, R. Kelly, G. Heaney, P. Rayhorn, C. Reid, K. J. Simon, G. S. Horan, N. Tao, H. A. Gardner, M. M. Skelly, A. M. Gown, G. J. Thomas, P. H. Weinreb, S. E. Fawell and S. M. Violette: Antibody-mediated blockade of integrin alpha v beta 6 inhibits tumor progression *in vivo* by a transforming growth factor-beta-regulated mechanism. *Cancer Res*, 68(2), 561-70 (2008)

70. S. C. Mok, K. K. Wong, R. K. Chan, C. C. Lau, S. W. Tsao, R. C. Knapp and R. S. Berkowitz: Molecular cloning of differentially expressed genes in human epithelial ovarian cancer. *Gynecol Oncol*, 52(2), 247-52 (1994)

71. S. C. Mok, W. Y. Chan, K. K. Wong, K. K. Cheung, C. C. Lau, S. W. Ng, A. Baldini, C. V. Colitti, C. O. Rock and R. S. Berkowitz: DOC-2, a candidate tumor suppressor gene in human epithelial ovarian cancer. *Oncogene*, 16(18), 2381-7 (1998)

72. Z. Fazili, W. Sun, S. Mittelstaedt, C. Cohen and X. X. Xu: Disabled-2 inactivation is an early step in ovarian tumorigenicity. *Oncogene*, 18(20), 3104-13 (1999)

73. S. A. Bagadi, C. P. Prasad, A. Srivastava, R. Prashad, S. D. Gupta and R. Ralhan: Frequent loss of Dab2 protein and infrequent promoter hypermethylation in breast cancer. *Breast Cancer Res Treat*, 104(3), 277-86 (2007)

74. J. A. Karam, S. F. Shariat, H. Y. Huang, R. C. Pong, R. Ashfaq, E. Shapiro, Y. Lotan, A. I. Sagalowsky, X. R. Wu and J. T. Hsieh: Decreased DOC-2/DAB2 expression in urothelial carcinoma of the bladder. *Clin Cancer Res*, 13(15 Pt 1), 4400-6 (2007)

75. Y. Huang, H. Friess, J. Kleeff, I. Esposito, Z. Zhu, S. Liu, S. C. Mok, A. Zimmermann and M. W. Buchler: Doc-2/hDab2 expression is up-regulated in primary pancreatic cancer but reduced in metastasis. *Lab Invest*, 81(6), 863-73 (2001)

76. J. Kleeff, Y. Huang, S. C. Mok, A. Zimmermann, H. Friess and M. W. Buchler: Down-regulation of DOC-2 in colorectal cancer points to its role as a tumor suppressor in this malignancy. *Dis Colon Rectum*, 45(9), 1242-8 (2002)

77. D. H. Yang, Z. Fazili, E. R. Smith, K. Q. Cai, A. Klein-Szanto, C. Cohen, I. R. Horowitz and X. X. Xu: Disabled-2 heterozygous mice are predisposed to endometrial and ovarian tumorigenesis and exhibit sex-biased embryonic lethality in a p53-null background. *Am J Pathol*, 169(1), 258-67 (2006)

78. X. Zhou and K. Chada: HMGI family proteins: architectural transcription factors in mammalian development and cancer. *Keio J Med*, 47(2), 73-7 (1998)

79. R. Reeves and L. Beckerbauer: HMGI/Y proteins: flexible regulators of transcription and chromatin structure. *Biochim Biophys Acta*, 1519(1-2), 13-29 (2001)

80. P. Rogalla, K. Drechsler, G. Frey, Y. Hennig, B. Helmke, U. Bonk and J. Bullerdiek: HMGI-C expression patterns in human tissues. Implications for the genesis of frequent mesenchymal tumors. *Am J Pathol*, 149(3), 775-9 (1996)

81. E. F. Schoenmakers, S. Wanschura, R. Mols, J. Bullerdiek, H. Van den Berghe and W. J. Van de Ven: Recurrent rearrangements in the high mobility group protein gene, HMGI-C, in benign mesenchymal tumours. *Nat Genet*, 10(4), 436-44 (1995)

82. B. Staats, U. Bonk, S. Wanschura, P. Hanisch, E. F. Schoenmakers, W. J. Van de Ven, S. Bartnitzke and J. Bullerdiek: A fibroadenoma with a t(4;12) (q27;q15)

affecting the HMGI-C gene, a member of the high mobility group protein gene family. *Breast Cancer Res Treat*, 38(3), 299-303 (1996)

83. J. M. Geurts, E. F. Schoenmakers, E. Roijer, A. K. Astrom, G. Stenman and W. J. van de Ven: Identification of NFIB as recurrent translocation partner gene of HMGIC in pleomorphic adenomas. *Oncogene*, 16(7), 865-72 (1998)

84. P. Dal Cin, S. Wanschura, B. Kazmierczak, G. Tallini, A. Dei Tos, J. Bullerdiek, I. Van den Berghe, P. Moerman and H. Van den Berghe: Amplification and expression of the HMGIC gene in a benign endometrial polyp. *Genes Chromosomes Cancer*, 22(2), 95-9 (1998)

85. M. R. Zaidi, Y. Okada and K. K. Chada: Misexpression of full-length HMGA2 induces benign mesenchymal tumors in mice. *Cancer Res*, 66(15), 7453-9 (2006)

86. J. V. Bokhman: Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol*, 15(1), 10-7 (1983)

87. M. E. Sherman, S. Sturgeon, L. A. Brinton, N. Potischman, R. J. Kurman, M. L. Berman, R. Mortel, L. B. Twiggs, R. J. Barrett and G. D. Wilbanks: Risk factors and hormone levels in patients with serous and endometrioid uterine carcinomas. *Mod Pathol*, 10(10), 963-8 (1997)

88. J. I. Risinger, A. Berchuck, M. F. Kohler, P. Watson, H. T. Lynch and J. Boyd: Genetic instability of microsatellites in endometrial carcinoma. *Cancer Res*, 53(21), 5100-3 (1993)

89. B. M. Ronnett, R. T. Burks, K. R. Cho and L. Hedrick: DCC genetic alterations and expression in endometrial carcinoma. *Mod Pathol*, 10(1), 38-46 (1997)

90. H. Tashiro, M. S. Blazes, R. Wu, K. R. Cho, S. Bose, S. I. Wang, J. Li, R. Parsons and L. H. Ellenson: Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. *Cancer Res*, 57(18), 3935-40 (1997)

91. J. I. Risinger, K. Hayes, G. L. Maxwell, M. E. Carney, R. K. Dodge, J. C. Barrett and A. Berchuck: PTEN mutation in endometrial cancers is associated with favorable clinical and pathologic characteristics. *Clin Cancer Res*, 4(12), 3005-10 (1998)

92. J. I. Risinger, A. K. Hayes, A. Berchuck and J. C. Barrett: PTEN/MMAC1 mutations in endometrial cancers. *Cancer Res*, 57(21), 4736-8 (1997)

93. H. B. Salvesen, I. Stefansson, E. I. Kretzschmar, P. Gruber, N. D. MacDonald, A. Ryan, I. J. Jacobs, L. A. Akslen and S. Das: Significance of PTEN alterations in endometrial carcinoma: a population-based study of mutations, promoter methylation and PTEN protein expression. *Int J Oncol*, 25(6), 1615-23 (2004)

94. R. F. Caduff, C. M. Johnston and T. S. Frank: Mutations of the Ki-ras oncogene in carcinoma of the endometrium. *Am J Pathol*, 146(1), 182-8 (1995)

95. H. Lagarda, L. Catasus, R. Arguelles, X. Matias-Guiu and J. Prat: K-ras mutations in endometrial carcinomas with microsatellite instability. *J Pathol*, 193(2), 193-9 (2001)

96. S. F. Lax, B. Kendall, H. Tashiro, R. J. Slebos and L. Hedrick: The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways. *Cancer*, 88(4), 814-24 (2000)

97. R. Halperin, S. Zehavi, L. Habler, E. Hadas, I. Bukovsky and D. Schneider: Comparative immunohistochemical study of endometrioid and serous papillary carcinoma of endometrium. *Eur J Gynaecol Oncol*, 22(2), 122-6 (2001)

98. A. Berchuck, G. Rodriguez, R. B. Kinney, J. T. Soper, R. K. Dodge, D. L. Clarke-Pearson and R. C. Bast, Jr.: Overexpression of HER-2/neu in endometrial cancer is associated with advanced stage disease. *Am J Obstet Gynecol*, 164(1 Pt 1), 15-21 (1991)

99. C. D. Rolitsky, K. S. Theil, V. R. McGaughy, L. J. Copeland and T. H. Niemann: HER-2/neu amplification and overexpression in endometrial carcinoma. *Int J Gynecol Pathol*, 18(2), 138-43 (1999)

100. D. Tritz, M. Pieretti, S. Turner and D. Powell: Loss of heterozygosity in usual and special variant carcinomas of the endometrium. *Hum Pathol*, 28(5), 607-12 (1997)

101. G. L. Mutter, M. C. Lin, J. T. Fitzgerald, J. B. Kum, J. P. Baak, J. A. Lees, L. P. Weng and C. Eng: Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. *J Natl Cancer Inst*, 92(11), 924-30 (2000)

102. H. Sun, T. Enomoto, M. Fujita, H. Wada, K. Yoshino, K. Ozaki, T. Nakamura and Y. Murata: Mutational analysis of the PTEN gene in endometrial carcinoma and hyperplasia. *Am J Clin Pathol*, 115(1), 32-8 (2001)

103. D. S. Charnock-Jones, A. M. Sharkey, P. Fenwick and S. K. Smith: Leukaemia inhibitory factor mRNA concentration peaks in human endometrium at the time of implantation and the blastocyst contains mRNA for the receptor at this time. *J Reprod Fertil*, 101(2), 421-6 (1994)

104. T. Ota, Y. Suzuki, T. Nishikawa, T. Otsuki, T. Sugiyama, R. Irie, A. Wakamatsu, K. Hayashi, H. Sato, K. Nagai, K. Kimura, H. Makita, M. Sekine, M. Obayashi, T. Nishi, T. Shibahara, T. Tanaka, S. Ishii, J. Yamamoto, K. Saito, Y. Kawai, Y. Isono, Y. Nakamura, K. Nagahari, K. Murakami, T. Yasuda, T. Iwayanagi, M. Wagatsuma, A. Shiratori, H. Sudo, T. Hosoiri, Y. Kaku, H. Kodaira, H. Kondo, M. Sugawara, M. Takahashi, K. Kanda, T. Yokoi, T. Furuya, E. Kikkawa, Y. Omura, K. Abe, K. Kamihara, N. Katsuta, K. Sato, M. Tanikawa, M. Yamazaki, K. Ninomiya, T. Ishibashi, H. Yamashita, K. Murakawa, K. Fujimori, H. Tanai, M. Kimata, M. Watanabe, S. Hiraoka, Y. Chiba, S. Ishida, Y. Ono, S. Takiguchi, S. Watanabe, M.

Yosida, T. Hotuta, J. Kusano, K. Kanehori, A. Takahashi-Fujii, H. Hara, T. O. Tanase, Y. Nomura, S. Togiya, F. Komai, R. Hara, K. Takeuchi, M. Arita, N. Imose, K. Musashino, H. Yuuki, A. Oshima, N. Sasaki, S. Aotsuka, Y. Yoshikawa, H. Matsunawa, T. Ichihara, N. Shiohata, S. Sano, S. Moriya, H. Momiyama, N. Satoh, S. Takami, Y. Terashima, O. Suzuki, S. Nakagawa, A. Senoh, H. Mizoguchi, Y. Goto, F. Shimizu, H. Wakebe, H. Hishigaki, T. Watanabe, A. Sugiyama, M. Takemoto, B. Kawakami, M. Yamazaki, K. Watanabe, A. Kumagai, S. Itakura, Y. Fukuzumi, Y. Fujimori, M. Komiyama, H. Tashiro, A. Tanigami, T. Fujiwara, T. Ono, K. Yamada, Y. Fujii, K. Ozaki, M. Hirao, Y. Ohmori, A. Kawabata, T. Hikiji, N. Kobatake, H. Inagaki, Y. Ikema, S. Okamoto, R. Okitani, T. Kawakami, S. Noguchi, T. Itoh, K. Shigeta, T. Senba, K. Matsumura, Y. Nakajima, T. Mizuno, M. Morinaga, M. Sasaki, T. Togashi, M. Oyama, H. Hata, M. Watanabe, T. Komatsu, J. Mizushima-Sugano, T. Satoh, Y. Shirai, Y. Takahashi, K. Nakagawa, K. Okumura, T. Nagase, N. Nomura, H. Kikuchi, Y. Masuho, R. Yamashita, K. Nakai, T. Yada, Y. Nakamura, O. Ohara, T. Isogai and S. Sugano: Complete sequencing and characterization of 21,243 full-length human cDNAs. *Nat Genet*, 36(1), 40-5 (2004)

105. M. Tanaka, S. Kyo, T. Kanaya, N. Yatabe, M. Nakamura, Y. Maida, M. Okabe and M. Inoue: Evidence of the monoclonal composition of human endometrial epithelial glands and mosaic pattern of clonal distribution in luminal epithelium. *Am J Pathol*, 163(1), 295-301 (2003)

106. C. Bilbao, G. Rodriguez, R. Ramirez, O. Falcon, L. Leon, R. Chirino, J. F. Rivero, O. Falcon, Jr., B. N. Diaz-Chico, J. C. Diaz-Chico and M. Perucho: The relationship between microsatellite instability and PTEN gene mutations in endometrial cancer. *Int J Cancer*, 119(3), 563-70 (2006)

107. K. Podsypanina, L. H. Ellenson, A. Nemes, J. Gu, M. Tamura, K. M. Yamada, C. Cordon-Cardo, G. Catoretti, P. E. Fisher and R. Parsons: Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. *Proc Natl Acad Sci U S A*, 96(4), 1563-8 (1999)

108. A. Suzuki, J. L. de la Pompa, V. Stambolic, A. J. Elia, T. Sasaki, I. del Barco Barrantes, A. Ho, A. Wakeham, A. Itie, W. Khoo, M. Fukumoto and T. W. Mak: High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. *Curr Biol*, 8(21), 1169-78 (1998)

109. A. Vilgelm, Z. Lian, H. Wang, S. L. Beauparlant, A. Klein-Szanto, L. H. Ellenson and A. Di Cristofano: Akt-mediated phosphorylation and activation of estrogen receptor alpha is required for endometrial neoplastic transformation in Pten+/- mice. *Cancer Res*, 66(7), 3375-80 (2006)

110. V. Stambolic, M. S. Tsao, D. Macpherson, A. Suzuki, W. B. Chapman and T. W. Mak: High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in pten+/- mice. *Cancer Res*, 60(13), 3605-11 (2000)

111. A. Staebler, S. F. Lax and L. H. Ellenson: Altered expression of hMLH1 and hMSH2 protein in endometrial carcinomas with microsatellite instability. *Hum Pathol*, 31(3), 354-8 (2000)

112. H. Wang, W. Douglas, M. Lia, W. Edelmann, R. Kucherlapati, K. Podsypanina, R. Parsons and L. H. Ellenson: DNA mismatch repair deficiency accelerates endometrial tumorigenesis in Pten heterozygous mice. *Am J Pathol*, 160(4), 1481-6 (2002)

113. H. J. Lee, H. Sakamoto, H. Luo, M. E. Skaznik-Wikiel, A. M. Friel, T. Niikura, J. C. Tilly, Y. Niikura, R. Klein, A. K. Styer, L. R. Zukerberg, J. L. Tilly and B. R. Rueda: Loss of CABLES1, a cyclin-dependent kinase-interacting protein that inhibits cell cycle progression, results in germline expansion at the expense of oocyte quality in adult female mice. *Cell Cycle*, 6(21), 2678-84 (2007)

114. T. Daikoku, Y. Hirota, S. Tranguch, A. R. Joshi, F. J. DeMayo, J. P. Lydon, L. H. Ellenson and S. K. Dey: Conditional loss of uterine Pten unfailingly and rapidly induces endometrial cancer in mice. *Cancer Res*, 68(14), 5619-27 (2008)

115. A. Fyles, G. Wood, M. Li, A. S. Manoukian, K. Gowing, R. Khokha, W. Chapman and M. S. Tsao: Neither ovariectomy nor progestin treatment prevents endometrial neoplasia in pten+/- mice. *Gynecol Oncol*, 108(2), 395-401 (2008)

116. C. L. Wu, S. D. Kirley, H. Xiao, Y. Chuang, D. C. Chung and L. R. Zukerberg: Cables enhances cdk2 tyrosine 15 phosphorylation by Wee1, inhibits cell growth, and is lost in many human colon and squamous cancers. *Cancer Res*, 61(19), 7325-32 (2001)

117. R. L. Debernardo, R. D. Littell, H. Luo, L. R. Duska, E. Oliva, S. D. Kirley, M. P. Lynch, L. R. Zukerberg and B. R. Rueda: Defining the extent of Cables loss in endometrial cancer subtypes and its effectiveness as an inhibitor of cell proliferation in malignant endometrial Cells *in vitro* and *in vivo*. *Cancer Biol Ther*, 4(1) (2005)

118. L. R. Zukerberg, R. L. DeBernardo, S. D. Kirley, M. D'Apuzzo, M. P. Lynch, R. D. Littell, L. R. Duska, L. Boring and B. R. Rueda: Loss of cables, a cyclin-dependent kinase regulatory protein, is associated with the development of endometrial hyperplasia and endometrial cancer. *Cancer Res*, 64(1), 202-8 (2004)

119. A. Ylikorkala, E. Avizienyte, I. P. Tomlinson, M. Tiainen, S. Roth, A. Loukola, A. Hemminki, M. Johansson, P. Sistonen, D. Markie, K. Neale, R. Phillips, P. Zauber, T. Twama, J. Sampson, H. Jarvinen, T. P. Makela and L. A. Aaltonen: Mutations and impaired function of LKB1 in familial and non-familial Peutz-Jeghers syndrome and a sporadic testicular cancer. *Hum Mol Genet*, 8(1), 45-51 (1999)

120. A. F. Hezel and N. Bardeesy: LKB1; linking cell structure and tumor suppression. *Oncogene*, 27(55), 6908-19 (2008)

121. A. Ylikorkala, D. J. Rossi, N. Korsisaari, K. Luukko, K. Alitalo, M. Henkemeyer and T. P. Makela: Vascular abnormalities and deregulation of VEGF in Lkb1-deficient mice. *Science*, 293(5533), 1323-6 (2001)

122. C. M. Contreras, S. Gurumurthy, J. M. Haynie, L. J. Shirley, E. A. Akbay, S. N. Wingo, J. O. Schorge, R. R. Broaddus, K. K. Wong, N. Bardeesy and D. H. Castrillon: Loss of Lkb1 provokes highly invasive endometrial adenocarcinomas. *Cancer Res*, 68(3), 759-66 (2008)

123. I. Miyoshi, K. Takahashi, Y. Kon, T. Okamura, Y. Mototani, Y. Araki and N. Kasai: Mouse transgenic for murine oviduct-specific glycoprotein promoter-driven simian virus 40 large T-antigen: tumor formation and its hormonal regulation. *Mol Reprod Dev*, 63(2), 168-76 (2002)

124. K. Jishage, J. Nezu, Y. Kawase, T. Iwata, M. Watanabe, A. Miyoshi, A. Ose, K. Habu, T. Kake, N. Kamada, O. Ueda, M. Kinoshita, D. E. Jenne, M. Shimane and H. Suzuki: Role of Lkb1, the causative gene of Peutz-Jegher's syndrome, in embryogenesis and polyposis. *Proc Natl Acad Sci U S A*, 99(13), 8903-8 (2002)

125. D. J. Rossi, A. Ylikorkala, N. Korsisaari, R. Salovaara, K. Luukko, V. Launonen, M. Henkemeyer, A. Ristimaki, L. A. Aaltonen and T. P. Makela: Induction of cyclooxygenase-2 in a mouse model of Peutz-Jeghers polyposis. *Proc Natl Acad Sci U S A*, 99(19), 12327-32 (2002)

126. A. Hemminki, D. Markie, I. Tomlinson, E. Avizienyte, S. Roth, A. Loukola, G. Bignell, W. Warren, M. Aminoff, P. Hoglund, H. Jarvinen, P. Kristo, K. Pelin, M. Ridanpaa, R. Salovaara, T. Toro, W. Bodmer, S. Olschwang, A. S. Olsen, M. R. Stratton, A. de la Chapelle and L. A. Aaltonen: A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature*, 391(6663), 184-7 (1998)

127. D. R. Alessi, K. Sakamoto and J. R. Bayascas: LKB1-dependent signaling pathways. *Annu Rev Biochem*, 75, 137-63 (2006)

128. A. M. Westerman, M. M. Entius, E. de Baar, P. P. Boor, R. Koole, M. L. van Velthuysen, G. J. Offerhaus, D. Lindhout, F. W. de Rooij and J. H. Wilson: Peutz-Jeghers syndrome: 78-year follow-up of the original family. *Lancet*, 353(9160), 1211-5 (1999)

129. W. Lim, N. Hearle, B. Shah, V. Murday, S. V. Hodgson, A. Lucassen, D. Eccles, I. Talbot, K. Neale, A. G. Lim, J. O'Donohue, A. Donaldson, R. C. Macdonald, I. D. Young, M. H. Robinson, P. W. Lee, B. J. Stoodley, I. Tomlinson, D. Alderson, A. G. Holbrook, S. Vyas, E. T. Swarbrick, A. A. Lewis, R. K. Phillips and R. S. Houlston: Further observations on LKB1/STK11 status and cancer risk in Peutz-Jeghers syndrome. *Br J Cancer*, 89(2), 308-13 (2003)

130. W. Lim, S. Olschwang, J. J. Keller, A. M. Westerman, F. H. Menko, L. A. Boardman, R. J. Scott, J. Trimbath, F. M. Giardiello, S. B. Gruber, J. J. Gille, G. J. Offerhaus, F. W. de Rooij, J. H. Wilson, A. D. Spigelman, R. K. Phillips and R. S. Houlston: Relative frequency and morphology of cancers in STK11 mutation carriers. *Gastroenterology*, 126(7), 1788-94 (2004)

131. K. Inoki, T. Zhu and K. L. Guan: TSC2 mediates cellular energy response to control cell growth and survival. *Cell*, 115(5), 577-90 (2003)

132. Y. M. Jonkers, S. M. Claessen, A. Perren, S. Schmid, P. Komminoth, A. A. Verhofstad, L. J. Hofland, R. R. de Krijger, P. J. Slootweg, F. C. Ramaekers and E. J. Speel: Chromosomal instability predicts metastatic disease in patients with insulinomas. *Endocr Relat Cancer*, 12(2), 435-47 (2005)

133. A. Semczuk, C. Boltze, B. Marzec, A. Szczygielska, A. Roessner and R. Schneider-Stock: p16INK4A alterations are accompanied by aberrant protein immunostaining in endometrial carcinomas. *J Cancer Res Clin Oncol*, 129(10), 589-96 (2003)

134. S. F. Sells, S. S. Han, S. Muthukkumar, N. Maddiwar, R. Johnstone, E. Boghaert, D. Gillis, G. Liu, P. Nair, S. Monnig, P. Collini, M. P. Mattson, V. P. Sukhatme, S. G. Zimmer, D. P. Wood, Jr., J. W. McRoberts, Y. Shi and V. M. Rangnekar: Expression and function of the leucine zipper protein Par-4 in apoptosis. *Mol Cell Biol*, 17(7), 3823-32 (1997)

135. I. Garcia-Cao, A. Duran, M. Collado, M. J. Carrascosa, J. Martin-Caballero, J. M. Flores, M. T. Diaz-Meco, J. Moscat and M. Serrano: Tumour-suppression activity of the proapoptotic regulator Par4. *EMBO Rep*, 6(6), 577-83 (2005)

136. J. Cook, S. Krishnan, S. Ananth, S. F. Sells, Y. Shi, M. M. Walther, W. M. Linehan, V. P. Sukhatme, M. H. Weinstein and V. M. Rangnekar: Decreased expression of the pro-apoptotic protein Par-4 in renal cell carcinoma. *Oncogene*, 18(5), 1205-8 (1999)

137. G. Moreno-Bueno, P. J. Fernandez-Marcos, M. Collado, M. J. Tendero, S. M. Rodriguez-Pinilla, I. Garcia-Cao, D. Hardisson, M. T. Diaz-Meco, J. Moscat, M. Serrano and J. Palacios: Inactivation of the candidate tumor suppressor par-4 in endometrial cancer. *Cancer Res*, 67(5), 1927-34 (2007)

138. R. A. DePinho: The age of cancer. *Nature*, 408(6809), 248-54 (2000)

139. P. Yan, J. Benhattar, J. M. Coindre and L. Guillou: Telomerase activity and hTERT mRNA expression can be heterogeneous and does not correlate with telomere length in soft tissue sarcomas. *Int J Cancer*, 98(6), 851-6 (2002)

140. K. K. Wong and R. A. DePinho: Walking the telomere plank into cancer. *J Natl Cancer Inst*, 95(16), 1184-6 (2003)

141. D. Gisselsson, T. Jonson, A. Petersen, B. Strombeck, P. Dal Cin, M. Hoglund, F. Mitelman, F. Mertens and N. Mandahl: Telomere dysfunction triggers extensive DNA

fragmentation and evolution of complex chromosome abnormalities in human malignant tumors. *Proc Natl Acad Sci U S A*, 98(22), 12683-8 (2001)

142. E. A. Akbay, C. M. Contreras, S. A. Perera, J. P. Sullivan, R. R. Broaddus, J. O. Schorge, R. Ashfaq, H. Saboorian, K. K. Wong and D. H. Castrillon: Differential roles of telomere attrition in type I and II endometrial carcinogenesis. *Am J Pathol*, 173(2), 536-44 (2008)

143. S. E. Artandi and R. A. DePinho: A critical role for telomeres in suppressing and facilitating carcinogenesis. *Curr Opin Genet Dev*, 10(1), 39-46 (2000)

144. S. E. Artandi and R. A. DePinho: Mice without telomerase: what can they teach us about human cancer? *Nat Med*, 6(8), 852-5 (2000)

145. D. W. Lee, T. B. Gibson, G. S. Carls, R. J. Ozminkowski, S. Wang and E. A. Stewart: Uterine fibroid treatment patterns in a population of insured women. *Fertil Steril* (2008)

146. P. E. Schwartz and M. G. Kelly: Malignant transformation of myomas: myth or reality? *Obstet Gynecol Clin North Am*, 33(1), 183-98, xii (2006)

147. U. Indraccolo, G. Luchetti and S. R. Indraccolo: Malignant transformation of uterine leiomyomata. *Eur J Gynaecol Oncol*, 29(5), 543-4 (2008)

148. S. E. Brooks, M. Zhan, T. Cote and C. R. Baquet: Surveillance, epidemiology, and end results analysis of 2677 cases of uterine sarcoma 1989-1999. *Gynecol Oncol*, 93(1), 204-8 (2004)

149. P. S. Tanwar, H. J. Lee, L. Zhang, L. R. Zukerberg, M. M. Taketo, B. R. Rueda and J. M. Teixeira: Constitutive activation of beta-catenin in uterine stroma and smooth muscle leads to the development of mesenchymal tumors in mice. *Biol Reprod* (2009)

150. C. L. Walker, D. Hunter and J. I. Everitt: Uterine leiomyoma in the Eker rat: a unique model for important diseases of women. *Genes Chromosomes Cancer*, 38(4), 349-56 (2003)

151. B. Romagnolo, T. Molina, G. Leroy, C. Blin, A. Porteux, M. Thomasset, A. Vandewalle, A. Kahn and C. Perret: Estradiol-dependent uterine leiomyomas in transgenic mice. *J Clin Invest*, 98(3), 777-84 (1996)

152. K. Politi, A. Kljuic, M. Szabolcs, P. Fisher, T. Ludwig and A. Efstratiadis: 'Designer' tumors in mice. *Oncogene*, 23(8), 1558-65 (2004)

153. L. Strizzi, C. Bianco, M. Hirota, K. Watanabe, M. Mancino, S. Hamada, A. Raafat, S. Lawson, A. D. Ebert, A. D'Antonio, S. Losito, N. Normanno and D. S. Salomon: Development of leiomyosarcoma of the uterus in MMTV-CR-1 transgenic mice. *J Pathol*, 211(1), 36-44 (2007)

154. B. Seliger, M. J. Maeurer and S. Ferrone: Antigen-processing machinery breakdown and tumor growth. *Immunol Today*, 21(9), 455-64 (2000)

155. L. Van Kaer, P. G. Ashton-Rickardt, M. Eichelberger, M. Gaczynska, K. Nagashima, K. L. Rock, A. L. Goldberg, P. C. Doherty and S. Tonegawa: Altered peptidase and viral-specific T cell response in LMP2 mutant mice. *Immunity*, 1(7), 533-41 (1994)

156. Z. P. Cai, Z. Shen, L. Van Kaer and L. C. Becker: Ischemic preconditioning-induced cardioprotection is lost in mice with immunoproteasome subunit low molecular mass polypeptide-2 deficiency. *Faseb J*, 22(12), 4248-57 (2008)

157. T. Hayashi and D. L. Faustman: Development of spontaneous uterine tumors in low molecular mass polypeptide-2 knockout mice. *Cancer Res*, 62(1), 24-7 (2002)

158. T. Hayashi, Y. Kobayashi, A. Horiuchi, N. Hiraoka, N. Kubota, Y. Kanai, H. Aburatani, I. Konishi and K. Sano: Molecular mechanisms of uterine leiomyosarcomas: Involvement of defective LMP2 activation. *Med Hypotheses Res*, 4, 11-20 (2008)

159. L. Yamasaki, T. Jacks, R. Bronson, E. Goillot, E. Harlow and N. J. Dyson: Tumor induction and tissue atrophy in mice lacking E2F-1. *Cell*, 85(4), 537-48 (1996)

160. Z. Zhang, J. Li, L. E. Lantry, Y. Wang, R. W. Wiseman, R. A. Lubet and M. You: p53 transgenic mice are highly susceptible to 1, 2-dimethylhydrazine-induced uterine sarcomas. *Cancer Res*, 62(11), 3024-9 (2002)

161. K. Mitsumori, H. Onodera, T. Shimo, K. Yasuhara, H. Takagi, T. Koujitani, M. Hirose, C. Maruyama and S. Wakana: Rapid induction of uterine tumors with p53 point mutations in heterozygous p53-deficient CBA mice given a single intraperitoneal administration of N-ethyl-N-nitrosourea. *Carcinogenesis*, 21(5), 1039-42 (2000)

162. K. Mitsumori, T. Shimo, H. Onodera, H. Takagi, K. Yasuhara, T. Tamura, Y. Aoki, O. Nagata and M. Hirose: Modifying effects of ethinylestradiol but not methoxychlor on N-ethyl-N-nitrosourea-induced uterine carcinogenesis in heterozygous p53-deficient CBA mice. *Toxicol Sci*, 58(1), 43-9 (2000)

163. H. Suzuki, T. Chiba, M. Kobayashi, M. Takeuchi, T. Suzuki, A. Ichiyama, T. Ikenoue, M. Omata, K. Furuichi and K. Tanaka: IkappaBalpha ubiquitination is catalyzed by an SCF-like complex containing Skp1, cullin-1, and two F-box/WD40-repeat proteins, betaTrCP1 and betaTrCP2. *Biochem Biophys Res Commun*, 256(1), 127-32 (1999)

164. T. Cardozo and M. Pagano: The SCF ubiquitin ligase: insights into a molecular machine. *Nat Rev Mol Cell Biol*, 5(9), 739-51 (2004)

165. L. Busino, M. Donzelli, M. Chiesa, D. Guardavaccaro, D. Ganoth, N. V. Dorrello, A. Hershko, M. Pagano and G.

F. Draetta: Degradation of Cdc25A by beta-TrCP during S phase and in response to DNA damage. *Nature*, 426(6962), 87-91 (2003)

166. H. Matsuyama, Y. Pan, S. Yoshihiro, D. Kudren, K. Naito, U. S. Bergerheim and P. Ekman: Clinical significance of chromosome 8p, 10q, and 16q deletions in prostate cancer. *Prostate*, 54(2), 103-11 (2003)

167. F. Mertens, B. Johansson, M. Hoglund and F. Mitelman: Chromosomal imbalance maps of malignant solid tumors: a cytogenetic survey of 3185 neoplasms. *Cancer Res*, 57(13), 2765-80 (1997)

168. Y. Kudo, D. Guardavaccaro, P. G. Santamaria, R. Koyama-Nasu, E. Latres, R. Bronson, L. Yamasaki and M. Pagano: Role of F-box protein betaTrcp1 in mammary gland development and tumorigenesis. *Mol Cell Biol*, 24(18), 8184-94 (2004)

169. S. Orsulic, Y. Li, R. A. Soslow, L. A. Vitale-Cross, J. S. Gutkind and H. E. Varmus: Induction of ovarian cancer by defined multiple genetic changes in a mouse model system. *Cancer Cell*, 1(1), 53-62 (2002)

170. D. M. Dinulescu, T. A. Ince, B. J. Quade, S. A. Shafer, D. Crowley and T. Jacks: Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. *Nat Med*, 11(1), 63-70 (2005)

171. F. Sugiyama, K. Yagami and B. Paigen: Mouse models of blood pressure regulation and hypertension. *Curr Hypertens Rep*, 3(1), 41-8 (2001)

172. M. Yoshioka, T. Kayo, T. Ikeda and A. Koizumi: A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice. *Diabetes*, 46(5), 887-94 (1997)

173. M. L. Klebig, J. E. Wilkinson, J. G. Geisler and R. P. Woychik: Ectopic expression of the agouti gene in transgenic mice causes obesity, features of type II diabetes, and yellow fur. *Proc Natl Acad Sci U S A*, 92(11), 4728-32 (1995)

174. H. Ueda, H. Ikegami, E. Yamato, J. Fu, M. Fukuda, G. Shen, Y. Kawaguchi, K. Takekawa, Y. Fujioka, T. Fujisawa and *et al.*: The NSY mouse: a new animal model of spontaneous NIDDM with moderate obesity. *Diabetologia*, 38(5), 503-8 (1995)

175. H. Kikutani and S. Makino: The murine autoimmune diabetes model: NOD and related strains. *Adv Immunol*, 51, 285-322 (1992)

176. S. P. Shah, M. Kobel, J. Senz, R. D. Morin, B. A. Clarke, K. C. Wiegand, G. Leung, A. Zayed, E. Mehl, S. E. Kalloger, M. Sun, R. Giuliany, E. Yorida, S. Jones, R. Varhol, K. D. Swenerton, D. Miller, P. B. Clement, C. Crane, J. Madore, D. Provencher, P. Leung, A. DeFazio, J. Khattra, G. Turashvili, Y. Zhao, T. Zeng, J. N. Glover, B. Vanderhyden, C. Zhao, C. A. Parkinson, M. Jimenez-Linan, D. D. Bowtell, A. M. Mes-Masson, J. D. Brenton, S. A. Aparicio, N. Boyd, M. Hirst, C. B. Gilks, M. Marra and D. G. Huntsman: Mutation of FOXL2 in granulosa-cell tumors of the ovary. *N Engl J Med*, 360(26), 2719-29 (2009)

177. W. B. Growdon, S. L. Boisvert, S. Akhavanfard, E. Oliva, D. C. Dias-Santagata, S. Kojiro, N. S. Horowitz, A. J. Iafrate, D. R. Borger and B. R. Rueda: Decreased survival in EGFR gene amplified vulvar carcinoma. *Gynecol Oncol*, 111(2), 289-97 (2008)

Key Words: Mouse Models, Uterus, Endometrium, Cancer, Hyperplasis, Leiomyoma, Review

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