Chapter 3

The role of the tumour suppressor gene PTEN in the etiology of uterine soft tissue tumours

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1   Introduction

1.1 Background

Uterine sarcomas are uncommon genital tract cancers, staged using the International Federation of Gynecology and Obstetrics (FIGO) staging for endometrial cancer and classified using histological subtypes. These tumours are mainly treated by surgery, tend to be aggressive neoplasms and are often diagnosed post-operatively on the histology report.

Although uterine sarcomas are rare (between 2 and 10% of malignancies of the uterine corpus), together they account for between 20 and 30% of the deaths due to malignancies of the corpus (Lurain et al 1997). Despite our efforts to combine surgical therapy with radiation and chemotherapy, we have not been able to lower the mortality due to these tumors. Instead there have been suggestions in the literature that the incidence is increasing and with it the mortality rate. The well-documented difference in incidence and mortality between races also suggests serious previous underreporting in older publications in many parts of the world (Muthupei & Maluleke 1998; Silverberg et al 1990; Harlow et al 1986; Christopherson et al 1972).

Numerous studies suggest that estrogen and Tamoxifen play important roles in the pathogenesis of these tumors (Zelmanovicz et al 1998; Schwartz et al 1991; Bokhman 1983) and previous pelvic irradiation has been estimated to increase the risk for uterine sarcoma by about 5,38 times after 10 to 20 years (Lurain et al 1991), suggesting at least a theoretical risk for an iatrogenic increase in incidence. All these factors lead us to believe that the clinical importance of uterine sarcomas will increase over the next decades.

Uterine sarcoma is a histologically divers group of malignancies, with the two most common tumors the leiomyosarcomas (LMS) and the malignant Mullerian mixed tumors (MMMT) or carcinosarcomas (CS). Uterine leiomyomas and sarcomas originate from the endometrium and myometrium and share many of the same etiological factors. The classical risk factors for the development of uterine sarcomas are listed in table 3.1.

Many of these factors, including age and endogenous or exogenous hyperestrogenism are also shared to some extent by endometrial polyps, endometrial cancer and breast cancer. All these partially related tumours are definitely or potentially hormone responsive. However, many reports also suggest important differences between the different types of sarcomas. The histopathology and pathogenesis are completely different for the two groups of tumours. Leiomyosarcoma arise from smooth muscle fibres in the myometrium or, possibly, in a pre-existing leiomyoma.

It is calculated that only about 0,1% of leiomyomas undergo so-called "sarcomatous degeneration", changing the tumour into a leiomyosarcoma. Leiomyomas thus cannot be considered pre-malignant. Nevertheless, it was thought appropriate to use uterine leiomyoma (and specifically cellular leiomyoma) as the benign counterpart for this study. These two benign and malignant soft tissue tumours of the corpus uteri share both histological origin and many etiological factors.

Table 3.13: Classical risk factors for uterine leiomyomas and sarcomas.

Endogenous hyperestrogenism
Exogenous hyperestrogenism
Tamoxifen use
Hereditary factors
Black race
Co-existing leiomyomas
Advanced age
Previous pelvic radiation

On the other hand the cellular origin of carcinosarcomas has been hotly debated with the current consensus on a monoclonal histogenesis from a toti-potential cell of the endometrium (Guarino et al 1998; Gorai et al 2002). These epidemiological and histopathological data suggest a major overlap in the development of carcinosarcoma and endometrial carcinoma. Carcinosarcoma will therefore be compared on molecular level with endometrial hyperplasia and endometrial carcinomas.

PTEN involvement in the tumorigenesis of both benign and malignant uterine soft tissue tumours is a very real possibility in the light of the abovementioned etiological and histological overlap. It is also possible that PTEN could play a role later in the development of the tumour or in the late stage tumours only, suggesting a role not in carcinogenesis, but in dedifferentiation. This would mimic the type of involvement of PTEN found in glioblastoma (see chapter 1).

In an attempt to help clarify the role of the PTEN gene in the development and progression of uterine mesenchymal tumours, it was decided to study involvement of the gene in uterine leiomyomas and uterine sarcomas.

We were also interested in comparing the involvement of the gene in the different tumours. An important difference between leiomyomas and carcinosarcomas would support the theory that these tumours have completely different pathogenetic pathways. PTEN involvement in carcinosarcoma similar to that found in poorly differentiated endometrial carcinomas would also support the hypothesis that this tumour develops as an extremely poorly differentiated endometrial carcinoma.

Existing knowledge does not to any extent predict the involvement of PTEN in uterine leiomyosarcoma and leiomyoma. This topic was not studied before. It would be expected that the gene will not be involved as it is not considered an important role-player in other sarcomas.

1.2 Research questions and hypothesis

This chapter will focus on existing knowledge of molecular evidence regarding uterine soft tissue tumour genesis, on the histopathology and clinical features of these tumours. An attempt will be made to put the molecular evidence into perspective of existing knowledge on the other aspects.

Additionally, involvement of the tumour suppressor gene PTEN in subsets of patients with benign and malignant uterine soft tissue tumours will be tested.

It is hypothesized that the PTEN gene is involved in the formation of the different uterine soft tissue tumours. It is thus expected that PTEN mutations will

be found in uterine sarcomas and some differences between the tumour types is expected. It is postulated that the gene could be involved even in the benign counterpart, namely uterine leiomyomas.

The research questions for this study as listed in chapter 1 will be:

1. What role does PTEN gene mutation and pten protein inactivation play in the etiology of uterine leiomyomas and uterine sarcomas?

2. What is the frequency of PTEN mutations in uterine soft tissue tumours?

3. Can these mutations be predicted by the finding of micro-satellite instability?

4. Can these mutations also be shown in uterine leiomyomas?

5. How does PTEN mutations correlate with histological type, disease stage and grade?

6. How does the involvement of the PTEN gene differ between the different population groups in South Africa?

2   Literature overview

2.1 Genetic changes in soft tissue tumours of the uterus

2.1.1   Chromosomal abnormalities in uterine leiomyomas and uterine sarcomas

2.1.1.1   Cytogenetic changes in uterine leiomyomas and sarcomas

In the existing published studies on the cytogenetic make-up of uterine leiomyomas, a multitude of sporadic changes have been reported, with about 50% of leiomyomas displaying abnormal cytogenetics. The constant changes are shown in table 3.2.

Table 3.14: Constant changes in the cytogenetics of uterine leiomyomas and possibly affected genes.

Chromosomal change	Gene involved
Deletion (7) (q22, q32)	> 1 gene?

		Few authors
Deletion (10) (q22)	PTEN	have
Monosomy 10	PTEN	published
Translocation (12;14)	HMG-I, ER	cytogenetic
Also: X,1,3	FH-gene	findings in

uterine sarcomas. The involvement of chromosomes 1,7,10 and 11 has been reported (Laxman et al 1993). Genes that are implicated include the genes for the high mobility group proteins (HMG-I), PTEN, CYP17 and the fumarate hydratase gene.

An important overlap is thus seen between the two groups with cytogenetic changes not confined to the malignant tumours. Cytogenetic studies can play an important role in determining clonality and finding new candidate tumour suppressor and oncogenes.

Monoclonality is now accepted for individual leiomyomas, intravenous leiomyomatosis, metastatic leiomyomas (eg. pulmonary), carcinosarcomas and leiomyosarcomas (Gokaslan et al 2005; Fujii & Dinulescu 2005; Gorai et al 1997; Doll et al 2008; Sonoda et al 2000).

2.1.1.2 Alterations of ploidy in uterine sarcomas

Using flow cytometry, alterations in ploidy was studied and found to be frequent in uterine sarcomas. Malmstrom and colleagues found that aneuploidy was associated with high stage, poor differentiation and a poor outcome. It was also associated with high mitotic count and other histologic markers of proliferation and poor differentiation. In this study about 50% of tumours were diploid and these had a significantly better prognosis than the aneuploid tumours (Major et al 1993).

2.1.2 Involvement of specific genetic alterations in leiomyomas and leiomyosarcomas

Uterine sarcomas are rare neoplasms and relatively few studies have addressed specific genomic changes in these tumours.

2.1.2.1 High mobility group (HMG) proteins

This group of highly evolutionary conserved proteins is involved in DNA-binding. Elevated expression of these proteins is associated with cellular transformation. The most important genes involved are the HMGIC gene at 12q15 and the HMGIY at 6p21. Although these genes and their protein products are definitely involved in leiomyomas and other soft tissue tumours the precise mechanism remains uncertain and information is incomplete.

2.1.2.2 CYP17 polymorphism

The CYP17 gene encodes cytochrome P450c17a, which regulates 17alpha-hydroxylase and 17,20-lyase. The gene has two alleles, namely A1 and A2. The A2 alleles are associated with high estrogen and progestogen in premenopausal women. In a study done at the University of Pretoria on 89 African and 56 Caucasian women, the distribution of the alleles in the two groups and also polymorphism distribution was comparable. The CYP17 A2A2 genotype in African women was, however strongly associated with leiomyoma formation and A2A2 women had myomas of larger diameter (Amant et al 2004). The precise meaning and importance of this gene, allele distribution and polymorphisms of the gene is not fully understood and deserves further study.

2.1.2.3 Fumarate hydratase (FH)

The FH gene, also called "MCUL1", is situated on chromosome 1q42.3-43, consists of ten exons and is involved in the Krebs cycle. Recently it has been found to also function as a tumour suppressor gene in tissue. Germline mutations cause the syndrome called MCUL (multiple cutaneous and uterine leiomyomata), multiple leiomyoma - (ML) or Reed syndrome. It is also the cause of hereditary leiomyoma and renal cell cancer syndrome, called HLRCC. When germline mutations are homozygous, fumarate hydratase deficiency exists.

The involvement of the gene in sporadic common uterine leiomyomas was the focus of a recent study done at the University of Pretoria. The questions asked, were whether low penetrance variants of germline mutations could be the cause and what the involvement of somatic mutations can be. The results of the Pretoria study showed mutations in seven of 28 leiomyomas, two of ten mitotically active leiomyomas and none of 21 leiomyosarcomas.

A total of 3 missense mutations were found in 4 tumours, namely one germline mutation in exon 8 and one somatic mutation each in exons 8 and 10. One nonsense mutation was found in two tumours, namely a somatic mutation in exon 3. Four synonymous or same sense mutations were demonstrated. These mutations were in the coding region of the protein and the importance is unknown. One of these was found in a mitotically active leiomyoma, suggesting that the mutation is not really silent.

This study was conducted on the same tumours that were used for PTEN analysis, and the interaction of the two genes will be discussed. It was the first study to find mutations in the FH gene in sporadic leiomyomas and the first study to involve mainly Black patients. These results have not been published and further study is warranted.

2.1.2.4   K-ras, C-myc
The role of mutations in these proto-oncogenes in uterine sarcomas has only been studied on a limited scale and results are inconclusive (Fotiou et al 1992).

2.1.2.5   HER 2/*neu* or c-*erb*B-2 and Bcl-2
The proto-oncogene bcl-2 is an inhibitor of programmed cell death. It counteracts the action of p53, which induces apoptosis. Morsi and colleagues studied bcl-2 protein expression in normal endometrium. This group observed cyclical changes of bcl-2 expression in normal endometrium and decreased expression levels in hyperplasia and carcinoma (Morsi et al 2000).

Several groups have shown that expression levels correlate negatively with differentiation grade (Geisler et al 1998; Zheng et al 1996). The correlation of the bcl-2 activity with carcinogenesis is still incompletely understood. Ioffe et al (1998) observed that the sub-cellular location of the bcl-2 protein seems important and not the quantity.

2.1.2.6   P 53
The involvement of the p53-gene is usually determined by using p53 overexpression on immunohistochemistry. Abnormal protein products lead to overexpression, pointing towards genetic mutation and thus to involvement of this gene in malignant transformation.

Several groups have studied the expression level of the p53 protein in uterine sarcomas using this method. In uterine sarcomas, like in many other tumours, reports suggest that overexpression is common (Liu et al 1994) and that it correlates with advanced stage disease and recurrence.

2.1.2.7 Microsatellite instability (MSI)

Endometrial stromal sarcoma and carcinosarcomas originate from endometrial stroma and epithelium. Microsatellite instability is frequent in endometrial cancer, and abnormality in DNA repair mechanisms plays an important role in these tumours. Therefore our group was interested to study the frequency and role of microsatellite instability in various subtypes of uterine sarcoma and leiomyoma. It was also interesting for the study on the involvement of the PTEN gene to correlate the findings of the MSI to the PTEN mutation analysis.

The same tumours were therefore studied for both replication errors and PTEN mutations. The results have been published (Amant et al 2001a) and correlation with PTEN results will be discussed in the last chapter.

Loss of heterozygosity (LOH) for chromosome 10q has been shown by Quade and colleagues (1999) to be frequent in LMS. This finding supported the untested hypothesis that the PTEN gene plays an important role in neoplastic transformation in these tumours.

2.1.2.8 PTEN

Multiple interactions of the protein product on cellular level was described and discussed in chapter 1. These include the induction of chemosensitivity, inhibition of Bcl2 expression and interaction with estrogen receptor-alpha and androgen receptors.

The role of the PTEN tumour suppressor gene in endometrial carcinogenesis has been studied extensively, but the involvement of the gene in uterine sarcomas has not received the same attention.

PTEN mutations were the focus of the current study. A detailed literature review of the involvement of the PTEN gene in uterine soft tissue tumours will be discussed below (2.3).

Forty-seven cases of uterine sarcoma and twenty-two cases of mitotically active leiomyomas (MAL) were analysed in the current study using genetic mutation analysis. The details of this study will be discussed in the rest of this chapter. The findings will be interpreted and compared to that of previous findings, similar studies and with the involvement of this gene in other malignant tumours of the genital tract (chapter 5).

2.2. Histology, pathogenesis and prognosis of uterine soft tissue tumours

2.2.1 Uterine leiomyomas

Uterine leiomyomas are extremely common benign soft tissue tumours of the uterus. The histogenesis has not been completely established and these tumours may arise from mature or immature uterine mesenchymal cells.

Table 3.15: Clinical associations and risk factors for uterine leiomyomas

Adenomyosis	Endometrial hyperplasia and carcinoma
Obesity	Diabetes mellitus
Family history	Excessive menstruation
Older age	Non-Caucasian race
Infertility	Hyper-estrogenic states

Leiomyomas occur in between 5 and 25% of younger women and some series have demonstrated up to 50% incidence in uteri at autopsy. Risk factors and clinical parameters associated with the development of these tumours are often quoted but mostly unproven. Some of these are listed in table 3.3. Inheritance play a definite although undefined role, with both population group (African and Afro-American origin) and family history as important risk factors.

Histological parameters of differentiation and especially proliferation are used to differentiate leiomyoma variants from their malignant counterpart, namely leiomyosarcomas. These parameters, as well as the size of the original malignant tumour (Levenback et al 1996) predict malignant behaviour as measured by local and systemic recurrences relatively well. However, all studies with histological review have a significant number of changed diagnoses, indicating low

repeatability and significant over-diagnosis of malignancy in historical samples (Evans et al 1988; Levenback et al 1996).

The most widely accepted histological defining criteria for uterine leiomyosarcomas used today is that of Stanford (Bell et al 1994), which utilizes the degree of cytological atypia, mitotic index and coagulative tumour cell necrosis (CTCN) instead of mitotic index alone as previously propagated. This classification system was evaluated in an outcomes based study of 213 problematic uterine smooth muscle neoplasms and enables differentiation between mitotically active leiomyomas (called 'leiomyoma with increased mitotic index'), atypical uterine leiomyomas and the aggressive and malignant leiomyosarcomas. These criteria accurately predict malignant behaviour, while reducing the false diagnosis of malignancy.

Recently molecular markers raised interest as a means to differentiate leiomyomas from leiomyosarcomas. Useful predictors would correlate well with the histological parameters and with clinical behaviour. Flow cytometric parameters seem promising including ploidy and S-phase fraction, but no definitive molecular marker have been identified that can reliably differentiate between these tumours.

Another new immuno-marker which seems promising is CD10, which stains both normal myometrium and leiomyomas positive, but sarcomas negative (Chu et al 2001).

Clinical parameters and risk factors for the diagnosis of uterine sarcoma in a patient with a "myomatous uterus" are listed in table 3.4. Although used clinically, these risk factors are not reliable and have poor sensitivity and specificity.

Table 3.16: Risk factors for the diagnosis of uterine sarcoma in patients with an enlarged or "myomatous" uterus.

Prolapsed tumour
Increase in size
Necrotic or infected soft tissue tumour
Bleeding tumour

| Painful tumour |
| Unusual or mixed density on ultrasound |

2.2.2 Uterine sarcomas

Although uterine sarcomas are commonly defined as a group of malignancies arising from the mesenchymal or connective tissue elements of the uterus suggesting relative homogeneity of origin, these tumours differ widely in histological appearance and origin. The most commonly occurring tumours are carcinosarcomas (CS) or malignant Mullerian mixed tumours, which consist of epithelial and mesenchymal components (~48%) and leiomyosarcomas (LMS) that are pure mesenchymal tumors (~37%) (Levenback et al 1996).

The histogenesis of these two tumour types is probably completely different. Leiomyosarcoma seems to arise from smooth muscle fibres in the myometrium or the myometrial blood vessels, while the current consensus about carcinosarcoma is that it probably originates in the endometrial epithelium (Gorai et al 2002, Fujii et al 2000). Endometrial stromal sarcomas are also pure mesenchymal tumours, probably arising from the endometrial stroma.

Data on ethnic and racial differences have shown not only an increased incidence for all uterine sarcomas, but also a higher mortality in African and Afro-American women. (Mutupei & Maluleke 1998; Silverberg et al 1990). The risk is however different for the two tumours with a reported increase by 60% (1,6 times risk) for leiomyosarcoma, while the risk for CS is about 2,7 times increased in Black women as compared to White women (Harlow et al 1986). As described previously, these aggressive neoplasms also contribute a larger proportion of all uterine malignancies in Non-Caucasians because the incidence of lower risk endometrial carcinomas is lower in these population groups.

It is interesting that most tumors following pelvic irradiation are carcinosarcomas (Levenback et al 1996) and that many researchers report a recent increase in the incidence of these CS rather than LMS (Kahanpaa et al 1986, Major et al 1993). The latter finding correlates with the increase in the occurrence of endometrial cancer.

The most recent histological typing and classification system was published by Scully and co-authors on behalf of the WHO (1994) and is adapted in table 3.5.

The system classifies tumours as pure mesenchymal (among other scarce types also the more common endometrial stromal sarcomas and leiomyosarcomas) and as mixed epithelial and mesenchymal in origin. The latter group consists of tumours with one benign and one malignant part (adenosarcomas and carcinofibromas) and tumours with malignant epithelial and malignant stromal components (carcinosarcomas).

Table 3.17: `Histopathologic classification of uterine sarcomas` (adapted from Scully RE et al 1994).

Pure malignant mesenchymal uterine tumours

Endometrial stromal sarcoma	
	Low-grade
	High-grade
Leiomyosarcomas	
	Epitheloid
	Myxoid
Mixed endometrial stromal and smooth muscle tumours	
Other malignant soft tissue tumours	
	Homologous
	Heterologous

Mixed malignant epithelial-mesenchymal uterine tumours

Adenosarcoma	
	Homologous
	Heterologous
Carcinosarcoma	
	Homologous
	Heterologous
Carcinofibroma	

2.2.2.1 Leiomyosarcoma

Uterine leiomyosarcoma arise from the smooth muscle cells of the uterus and account for about 35% of uterine sarcomas. These tumours are known to have complex cytogenetic abnormalities and rarely originate from previously existing benign neoplasms like leiomyomas although the two tumour types are associated (Evans et al 1988). Generally leiomyosarcomas occur at a younger age than carcinosarcomas with a

peak incidence at around 45 years. The histological criteria for the diagnosis were discussed above (Bell et al 1994).

The widely quoted five year survival rate for uterine leiomyosarcomas of all risk groups together is ~50%. Various studies have suggested and denied many different prognostic factors, including mitotic rate, cellular atypia and age. Tumour size and extra-uterine spread (as reflected in FIGO stage) seem to be obvious prognostic factors and was confirmed in the large-scale study by Evans et al (1988).

2.2.2.2 Carcinosarcoma

These highly malignant tumours are the most common uterine sarcomas, comprising about 55% of the total. Both epithelial and stromal components are malignant and are classified according to type. The most common sarcomatous components are ESS, rhabdomyosarcomas and fibrosarcomas, while the epithelial part consists mainly of endometroid adeno-, serous papillary adeno-, squamous - and undifferentiated carcinoma.

Like in endometrial cancer, age is a significant risk factor for the development of CS, with the incidence rising with older age and the highest risk occurring in the age group above 65 years. Although single cases have been described before the age of 45, this tumour is not typically associated with women in their reproductive age.

It appears from numerous sources and publications that these tumours represent dedifferentiated epithelial neoplasms, probably originating from an early mutation in a pre-existing endometrial carcinoma cell. Clinical data, tumour behaviour patterns and molecular evidence supports the monoclonal theory of tumorigenesis and supports the hypothesis of the epithelial component being the first component from which the mesenchymal cells develop via cellular mutation. This process represents a monoclonal pathway of stepwise dedifferentiation starting from a multipotential cell (Guarino et al 1998; Gorai et al 2002).

Epidemiological and histopathological data also suggest a major overlap in the development of CS and endometrial carcinoma. The current study aims to test this theory in part by examining associations in molecular findings.

Studies utilizing x-inactivation suggests that a small proportion of these tumours originate not from one, but from two endometrial carcinomatous foci, suggesting the so-called "clash theory" in about 5% of the tumours (Zelmanowitz et al 1998).

These biphasic tumours are aggressive and resistant to available therapeutic modalities. The behaviour of the carcinomatous part of the tumour determines prognosis and tumour behaviour and will be found mostly in metastases. Carcinosarcoma has a poor prognosis with an overall survival rate of about 20%.

2.2.2.3 Endometrial stromal sarcoma

Endometrial stromal sarcomas (ESS) are derived from the stromal elements of the endometrium. Tumours from the endometrial stroma can vary from totally benign (endometrial stomal myosis) to highly malignant and poorly differentiated sarcomas (high grade ESS). All these tumours are uncommon and ESS make up only about 10% of the group of uterine sarcomas (Zaloudek & Norris 1994). Hormone responsiveness seems to be certain (like the tissue of origin), may be related to differentiation and reports have seen the light that suggested an improved outcome where initial surgery included adnexectomy.

Survival of all ESS tumours together is generally reported to be better than CS and LMS (Piura et al 1997b). However, survival of high grade ESS is similar to CS (~25% five year survival), while low-grade tumours have an excellent prognosis (Evans et al 1988). Benign variants never metastasize. Generally the clinical behaviour is thus predicted very accurately by histological differentiation, which differs from the predictability of the other uterine smooth muscle tumours.

Nordal and Thoresen (1997) also demonstrated a better overall survival rate for ESS than for the other uterine sarcomas.

2.3 The PTEN gene and uterine leiomyomas and sarcomas

2.3.1 PTEN in normal endometrium and myometrium

The expression of pten in the normal endometrium changes in response to hormonal variations. During the proliferative phase pten is expressed in all tissue types in the uterus, while expression is increased in the early secretory phase and

lowered in the late secretory phase. These changes seem to be confined to the functionally active and hormonally responsive layers of endometrium (Mutter et al 2000). PTEN expression in the endometrial stroma has not been studied.

PTEN expression is also present in normal myometrium and is higher than in even benign neoplasms (Kayisli et al 2007). Although similar levels of pten may be found using immuno-staining, phosphorylation may be very different ,influencing the function (Kovacs et al 2007).

These tests reflect differences in protein production and function, suggesting up- and down-regulation of the protein production. On the other hand somatic PTEN gene mutation is never associated with normal tissue.

2.3.2 Frequency of PTEN alterations in uterine leiomyomas and sarcomas

2.3.2.1 Germline mutations

As described in chapter 1, PTEN was initially found as a result of the mapping of the susceptibility gene for Cowden syndrome and has subsequently also been linked to the Banayan-Zonana and Proteus syndromes. Mice with pten protein knockout develop complex proliferative endometrial lesions pointing to importance in cellular growth regulation in the female reproductive tract (Podsypanina et al 1999). Germline mutations are very uncommon in sporadic endometrial cancer and are not suspected to be involved in sporadic uterine sarcomas and leiomyomas.

2.3.2.2 Somatic mutations

After the finding of LOH on chromosome ten in endometrial cancer, various reports have shown the PTEN or MMAC1 gene, located on 10q23-24 (Steck et al 1997; Li et al 1997a), to be the most commonly mutated tumour suppressor gene in endometrial carcinoma (Tashiro et al 1997; Risinger et al 1997; Kong et al 1997). Quade et al (1999) reported frequent loss of heterozygosity of the short arm of chromosome 10 in uterine leiomyosarcomas as well. These findings suggest that PTEN/MMAC1 inactivating mutations may also play a role in the tumorigenesis of uterine sarcomas.

Kayisli et al recently (2007) reported a study comparing pten expression (immunohistochemistry) in normal myometrium with that in leiomyoma. They found lower expression in leiomyomas using immunohistochemistry and interprets this as a possible involvement of the gene in suppressing apoptosis. This finding does not necessarily implicate genetic mutation and could also be a result of down-regulation via some pathway. These findings cannot be compared directly with the results of mutation analysis.

Using immuno-staining, other researchers found similar pten staining in leiomyoma, atypical myoma and leiomyosarcoma, suggesting no involvement of PTEN in the pathogenesis (Gokaslan et al 2005). Semczuk and co-workers reported similar findings (2008) in a single case report.

The incidence of somatic mutations in endometrial cancer is the highest of any primary malignancy analysed so far, with frequencies reported from 40% to 76% (Nagase et al 1996; Peiffer et al 1995; Risinger et al 1997; Tashiro et al 1997). If carcinosarcomas were thought to be derived from endometrial carcinoma, it would be logical to expect PTEN mutations also in these tumours. It would also then be interesting to compare these findings with mutation analysis of the same gene in leiomyosarcomas and its benign counterpart, leiomyoma. The role of the tumour suppressor gene in uterine ESS has never been studied and very little is known of the carcinogenesis and molecular biology of this tumour.

Although immunostaining became freely available, is easier, can be done on existing slides and is much cheaper, mutation analysis is still the gold standard. This method was thus chosen for the current study. In addition immunostaining is less reproducible than mutation analysis.

3. Materials and methods

3.1 Materials

3.1.1 Sampling and clinical material

All cases of uterine sarcoma diagnosed from 1990 to 2000 at the Pretoria Academic complex (81 cases) were evaluated for the purposes of this study. In all these patients, the diagnosis was made on histology. Criteria for inclusion in this

study were that the histological diagnosis of uterine sarcoma be confirmed and that the histological material be adequate for the purposes of the analysis. Clinical data was collected from the files of the gynaecologic oncology unit or hospital archive when necessary.

3.1.2 Histology reports

The original study group consisted of 47 leiomyosarcomas, 28 carcinosarcomas and six endometrial stromal sarcomas. After careful review of the histology, the following material was available for further analysis:

Twenty-one cases of leiomyosarcoma were reclassified after histological review. Eighteen tumours were classified as mitotically active uterine leiomyoma using the Stanford criteria published in 1994. One case of atypical leiomyoma was diagnosed and two cases of extrauterine LMS. Three cases were excluded due to other reasons, leaving 19 cases of uterine leiomyosarcoma for analysis. Twenty-eight cases of carcinosarcoma were evaluated and the histological diagnosis was confirmed in all cases, except for one case of pure rhabdomyosarcoma. Three additional cases were excluded for technical reasons, leaving 24 CS tumours for further analysis. All of the six original cases of uterine stromal sarcoma was confirmed and remained in the study. (Amant et al 2002a)

The 21 leiomyosarcoma tumours that were reclassified after histological review (most of them to mitotically active leiomyomas) were used as the benign counterpart of uterine sarcomas, postulating that these tumours represent the most "pre-malignant" subsection of the benign mesenchymal tumours. These tumours were thus also included in the study and full mutation analysis was carried out on this material. The theory that these tumours will therefore be most likely to harbour the typical mutations of the malignant counterpart has not been tested before.

3.1.3 Tissue for DNA analysis

Paraffin embedded tissue was retrieved from the pathology archive for analysis. In all cases tissue material from both the tumour and the normal uterine tissue (myometrium) was obtained. After DNA extraction, mutation analysis was done

on both the tissue samples and results were then correlated and compared with available clinical and pathological data.

3.2 Methods

Tumour and normal tissue of 49 cases of uterine sarcoma (19 LMS, 24 CS and six cases with ESS) was analysed. Twenty-one cases of the chosen benign counterpart were also analysed fully (both tumour and normal tissue).

3.2.1 DNA extraction

Micro-dissection of formalin-fixed paraffin-embedded normal and tumour tissue was carried out in collaboration with an anatomic pathologist. After hematoxylin staining, the pathologist indicated normal (myometrial) and tumour (sarcoma or leiomyoma) areas on one slide. This slide was used as a guide to remove normal and tumour tissue separately with a sterile blade from five to ten consecutive sections per patient.

The micro-dissected paraffin embedded tissues were transferred to a microfuge tube and 200μl of extraction buffer (10 mM Tris-HCl, pH 8,3; 50 mM KCl; 0,45% Nonidet P40 and 0,45% Tween20) added where-after it was digested overnight at 56°C with proteinase K (final concentration 200μg / ml). The proteinase K was inactivated by boiling for 10 minutes at 95°C, quenched on ice and spun down.

The resulting supernatant containing the DNA was transferred to new sterile tubes and used or stored at 5°C.

3.2.2 DNA amplification

PTEN-coding sequences were amplified by polymerase chain reaction using the primers described by Davies et al (1999a). The nine exons were amplified in eleven sections, with exons five in two sections and nine in two sections. Intron-based primers were used to minimise the risk of amplifying the processed PTEN pseudogene on chromosome 9, as previously discussed.

PCR was performed in 20μl or 10μl reaction volumes for first round, or second round reactions, respectively. First round reactions containing 4μl of the

tissue extract, 20mM Tris-HCl (pH8.4), 50mM KCl, MgCl$_2$ (1.5mM for exon 8b; 2mM for exons1-7; 2.5mM for exon 8a; 3mM for exon 9), 0.25μM of each dNTP, 0.2μM of each primer and 0.5 units *Taq* DNA Polymerase (Life Technologies, BRL) were amplified for 35cycles consisting of 1 min at 94°C, 1 min at annealing temperature, and 1 min at 72°C, with a final extension step at 72°C for seven minutes. The PCR products were labelled with γ-^{32}P ATP (7000Ci/mmol; ICN) in a second round reaction in which two μl of the first round reaction was amplified in the presence of 0.02 μM (0.42μ Ci) of each end-labelled primer. The primer sequences, the amplification conditions and product lengths are displayed in table 3.5 and are similar to those described in chapter 2.

3.2.3   PTEN mutation analysis

Samples of amplified DNA were screened for mutations using exon-by-exon SSCP analysis. All of the eleven PCR products (exons and parts of exons) that displayed aberrant bands were directly sequenced.

3.2.3.1 Single Strand Conformational Polymorphism

PCR products were diluted 1:10 with denaturing buffer, denatured at 95°C (5 minutes), quenced on ice and 3 μl product was loaded on a 0.5 X Mutation Detection Enhancement (MDE) gel. The gels were run at 8 Watts, 14-20 h in 0,6 X TBE buffer and read after drying using exposure to medical X-ray film (Fuji) as described in chapter 2.

Table 3.18: Primers and optimised conditions used for amplification and mutation detection by SSCP in uterine soft tissue tumours

Exon	Primer name	Primer sequence	Product length (bp)	PCR conditions: Temp (°C) MgCl2 (mM)	SSCP conditions (hours at 8W)
1	PTEN 1F PTEN 1R	caagtccagagccatttcc cccacgttctaagagagtga	233	58 2.0	16
2	PTEN 2F PTEN 2R	ttcttttagtttgattgctg gtatcttttctgtggcttag	239	50 2.0	16
3	PTEN 3F PTEN 3R	ctgtcttttggtttttctt caagcagataactttcactta	213	50 2.0	15
4	PTEN 4F PTEN 4R	tataaagattcaggcaatgtt cagtctatcgggtttaagtta	190	50 2.0	15
5A	PTEN 5AF PTEN 5AR	ttgttaattaaaaattcaagag gcacatatcattacaccagt	217	48 2.0	15
5B	PTEN 5BF	tgaccaatggctaagtgaa	248	50	16

	PTEN 5AR	aaaagaaacccaaaatctgtt		2.0	
6	PTEN 6F PTEN 6R	cccagttaccatagcaat taagaaaactgttccaataca	275	50 2.0	16
7	PTEN 7F PTEN 7R	ttgacagttaaaggcatttc cttattttggatatttctcc	264	50 2.0	16
8A	PTEN 8AF PTEN 8AR	ttcatttcttttttctttcttt ggttggctttgtctttctt	238	53 2.5	15,5
8B	PTEN 8BF PTEN 8BR	ccaggaccagaggaaac cacatacatacaagtcacaa	235	56 1.5	16
9	PTEN 9F PTEN 9R	agtcatatttgtgggtttt ttattttcatggtgttttatc	268	48 3.0	17

3.2.3.2 Sequence analysis

Direct DNA sequencing was performed using Sequenase PCR product Sequencing Kit (Amersham Life Sciences) as prescribed by the manufacturer. Sequenced samples were diluted and heat denatured and 3 μl was loaded on a 6% denaturing polyacrylamide gel. Electrophoresis was performed in 1X TBE buffer at 60 Watts and results read as described before.

4. Results

4.1 Clinical data

Data used in sections 4.4.1 and 4.4.2 is based in part on the clinico-pathological study done on the same patient population by Amant et al, published in the European Journal of Gynaecological Oncology (2001b).

4.1.1 Age distribution

The mean age at diagnosis of the nineteen patients with leiomyosarcoma was 57 years, of the twenty-four patients with carcinosarcoma it was 65 years and of the six women with endometrial stromal sarcoma it was about 55 years (Amant et al 2001b). This finding that carcinosarcoma occurred at a significantly higher age than the other sarcomas correlates with the findings of other reviews (Evans et al 1988; Muthuphei & Maluleke 1998).

The fact that CS also occurs in the elderly similar to endometrial carcinoma, supports the theory that this tumour represents a form of poorly differentiated endometrial carcinoma, rather than a myometrial tumour or a true primary mesenchymal tumour or sarcoma.

4.1.2 Menopausal status

According to the available data, about a third of the patients presenting with LMS and ESS were pre-menopausal at the time of diagnosis. In contrast to this finding, only one patient (~4%) with CS was pre-menopausal at the time of diagnosis. These data could be interpreted in the same way as the data on age distribution.

4.1.3 Stage distribution

In this study, about 25% of patients with leiomyosarcomas presented in FIGO stage I, about 25% in stage III, and around 30% in stage IV. FIGO stage II describes patients where the uterine tumour grows down into the uterine cervix, thus a stage highly unlikely for a solid tumour. The distribution pattern was almost identical for carcinosarcomas, with around 10% of patients with both these tumours unstaged on the information we had available. The stage distribution is shown in figure 3.1. Patients with endometrial stromal sarcoma presented with earlier stage disease, with more than 80% of tumours in stage I. Evans et al (1988) ascribe this phenomenon to the perception that these tumours cause uterine bleeding at an earlier stage and patients therefore present earlier. It is however also possible that these tumours remain at an earlier stage for longer and are thus diagnosed at an earlier stage. This finding could simply reflect the less aggressive tumour behaviour.

Clinical data on age and menopausal state for LMS and ESS corresponded well. On the other hand, the data on tumour behaviour (stage distribution) for LMS and CS correlated more closely, while ESS had more favourable clinical features. This suggests that some factors in the pathogenesis of LMS and ESS are similar but are not shared by CS. On the other hand the prognostic features of CS and LMS did not differ much, while the prognosis for ESS is better.

Figure 3.11: Stage distribution according to tumour type
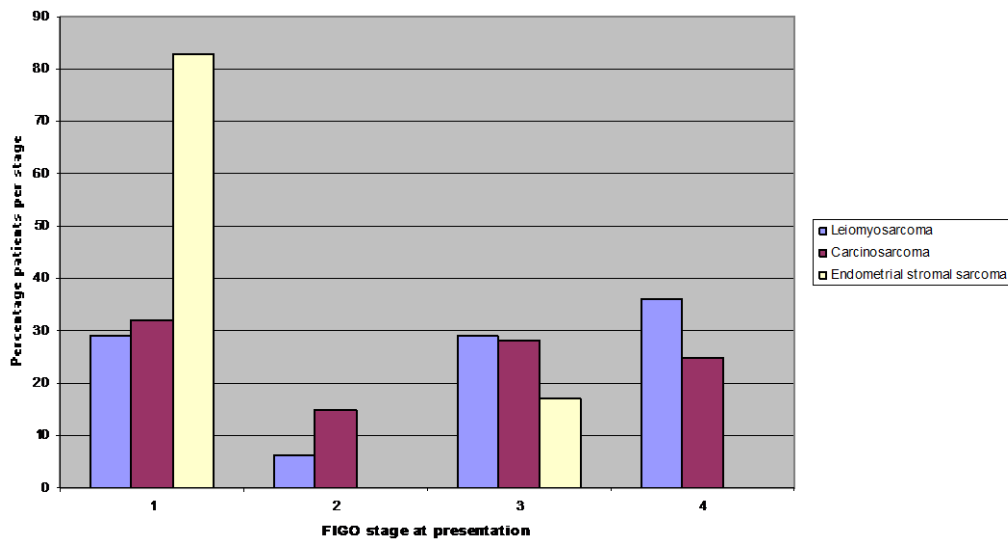
4.1.4 Symptoms

Vaginal bleeding is considered a symptom associated with either cervical or endometrial pathology. On the other hand general lower abdominal pain and the awareness of uterine enlargement are considered symptoms commonly caused by pressure or a mass. In our study significantly more patients with carcinosarcoma than leiomyosarcoma patients complained of endometrial disease type symptoms (~87% vs 53% had abnormal vaginal bleeding), while significantly more patients with leiomyosarcoma complained of pain or pressure symptoms (74% vs 29%).

We postulate that this difference reflects in part the difference in the origin and position of the tumours. See figure 3.2.

4.1.5 Differences between population groups

In an important population based study, Plaxe and Saltzstein reported that African and Afro-American women develop significantly less low risk tumours and in fact have the same incidence of high risk endometrial cancer subtypes (1997). Many other studies have confirmed this much higher risk for corpus carcinoma in Caucasian than other races, but not an important difference in total survival (Liu et al 1995; Schiff et al 1997), due to a higher proportion of high-risk tumours in mainly African races.

Uterine sarcomas have also been shown convincingly to have a higher incidence in Black women and higher mortality rates (Madison et al 1998; Arrastia et al 1997). Uterine sarcomas form a bigger proportion of all uterine malignancies in African populations, but it is unclarified whether this is due to a lower incidence of more indolent endometrial carcinomas or due to a truly higher incidence rate.

Figure 3.12: Symptoms according to tumour type.
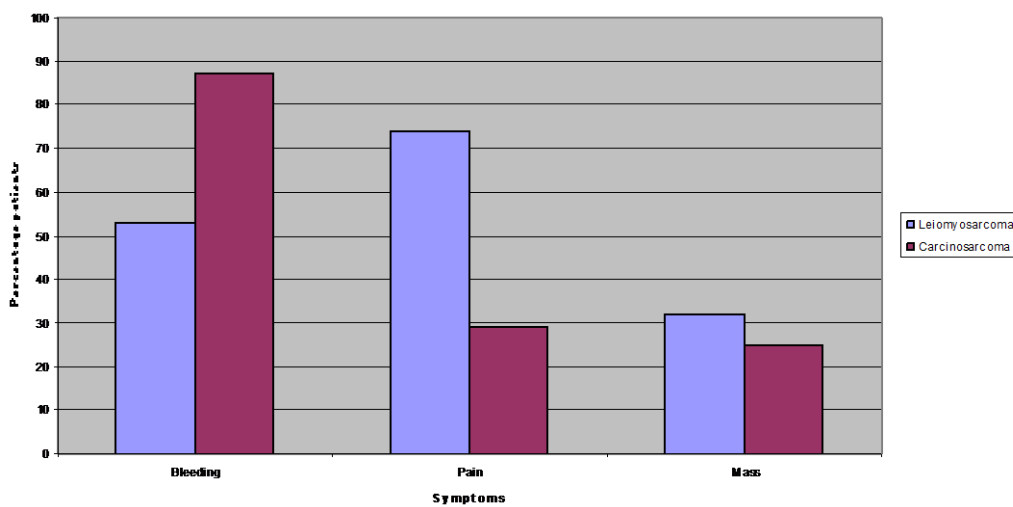
If carcinosarcoma were considered one of the poorly differentiated carcinomas these findings would suggest at least a similar incidence for this tumour in Black than in White females. The fact that these tumours occur at a much higher incidence in African and in Afro-American women is not yet well explained.

The incidence of these tumours and the outcome in different South African race groups has not been studied comprehensively or in a population-based way, but our study again suggests a higher incidence in Black women. Forty-three cases occurred in African patients, three in Caucasian patients (two carcinosarcomas and one leiomyosarcoma) (about 6%) and one case (LMS) in a coloured patient (mixed ancestry). The distribution pattern of endometrial cancer was about one third (~30%) Caucasian and two thirds African. For these two tumour types, the Unit will be expected to have a similar drainage area. This is a postulate.

It is widely accepted that leiomyoma occurs more commonly in African and Afro-American women. It is also well described that there is an association between leiomyoma and leiomyosarcoma, although the latter does not commonly develop from the first. It is therefore no surprise that leiomyosarcoma also seem to occur more often in Black than White women, as is the case in our study. The reasons for these disparities are, as discussed above, poorly understood.

4.2 Histology data

Seleye-Fubara and Uzoigwe (2007) showed that among uterine sarcomas carcinosarcoma was the most common (36%), followed by leiomyosarcoma. In their study 4% of all gynaecologic malignancies were uterine sarcomas.

In our review of nine years, carcinosarcoma was also the most common sarcoma (24 of 47) and represented 51%. This was followed by leimyosarcoma (19 patients, 40%) and endometrial stromal sarcoma (6 patients, 8%). We do not have accurate data on the total number of uterine malignancies over this period.

4.2.1 Leiomyosarcoma

The diagnostic criteria used in the review of histology slides were discussed above (3.1.2). Poor prognostic features on histology are included in the criteria for classification as a sarcoma and naturally would have a high incidence in the LMS tumours. These features include coagulative tumour cell necrosis (CTCN), lymphovascular invasion (LVS) and cellular atypia. Respectively these features were demonstrated in 16, seven and 18 of the 19 leiomyosarcomas. Additionally spread of the disease outside the uterus was histologically confirmed in some patients resulting in upstaging. This included spread to the omentum (two patients), adnexae (four patients), lymph nodes (two patients) and the intestines (also two patients).

4.2.2 Carcinosarcoma

Carcinosarcomas are poorly differentiated tumours and all 24 tumours displayed some poor prognostic features. Lymphovascular spread was present in the majority of tumours (13 of 24), coagulative tumour cell necrosis in 21 tumours (84%) and all tumours had cellular atypia. Extra-uterine spread was confirmed in the omentum in three cases, adnexae in six cases, while one case each had spread

to the lymph nodes, lung, intestines and peritoneum. The comparison with other tumour types are shown in figure 3.3.

The sarcomatous elements were also classified according to the Scully system quoted above (table 3.4) into homologous and heterologuous types. Three tumours had homologous sarcoma components, 17 had heterologous elements and four could not be classified.

4.2.3 Endometrial stromal sarcoma

Five of the six tumours were classified as high-grade tumours, with CTCN present in four of six patients and significant atypia in five. One patient had extra-uterine spread with ascites at the time of diagnosis. All others were diagnosed in FIGO stage I.

Figure 3.13: Poor prognostic features according to tumour type.
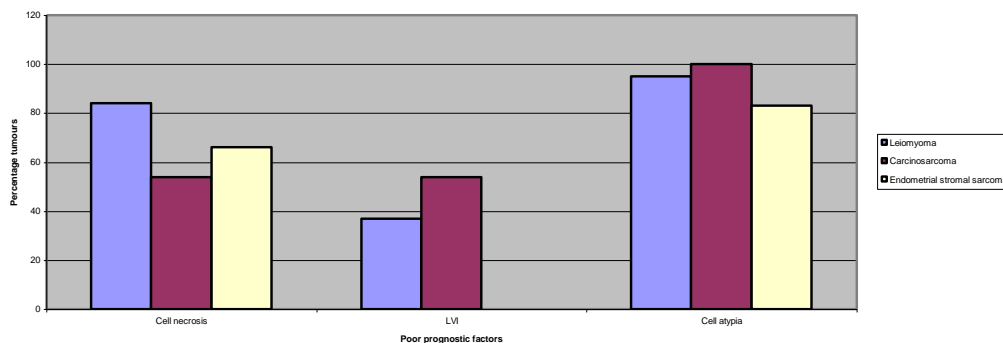
4.3 Mutation screening

4.3.1   Single Strand Conformational Polymorphism (SSCP) results

All aberrations on the screening test by SSCP were followed up by sequence analysis. The results will be discussed here.

4.4 Sequence analysis

4.4.1   Non-malignant tissue samples

No mutations were found in any of the twenty-one leiomyomas included in this study. A significant role for PTEN in this benign and potentially pre-malignant tumour type is therefore excluded by this study in spite of the small numbers.

All the samples of normal myometrium representing normal or germline DNA, tested negative for PTEN mutation as well. This excludes germline or inherited mutation as an underlying causative factor and confirms that mutations demonstrated in the tumour DNA are indeed novel somatic mutations, usually disease causing.

4.4.2 Leiomyosarcomas

One PTEN mutation was found in one of the nineteen leiomyosarcomas. This nonsense mutation is a C-to-T transition, resulting in protein truncation. The mutation was absent in the normal tissue (myometrium) confirming a somatic mutation. The mutation occurred in exon 5 and affected codon 130, which is a mutational hotspot (Myers et al 1997a; Bonneau & Longy 2000).

This mutation is considered definitely significant (disease causing) as codon 130 lies within the phosphatase core area and mutations here would affect the phosphatase activity of the protein product. The phosphatase activity of the pten-protein is the core of its tumour suppressor activity, as discussed in chapter 1.

Table 3.19: Mutations in the PTEN gene in leiomyosarcomas.

Tumour	Mutation type	Exon	Nucleotide change	Effect
LMS 42	nonsense	5	c.388C to T	Arg130Stop

In a separate study of micro-satellite instability and loss of heterozygosity on these same tumour samples, no MSI were demonstrated in LMS 42. Failure of post-replication repair does not seem to be an important factor in tumorigenesis in this tumour type. Neither was any focus of LOH identified to flag a possible novel gene that could be involved.

4.4.3 Carcinosarcomas

We were interested to see whether PTEN mutations could be demonstrated in carcinosarcomas with endometroid epithelial components, as this would support a shared etiopathogenesis with endometrial carcinoma.

We found four mutations in carcinosarcomas, all somatic with absence of mutations in the normal DNA represented by normal myometrium. This method

of including normal DNA in mutation analysis also guarded us from demonstrating artefact mutations (PCR-induced, pseudogene induced or mutations caused by the method of paraffin extraction) as all these are likely to also be present in the normal DNA.

All three tumours harbouring the four mutations had an endometroid adenocarcinoma epithelial component. In no CS with any other epithelial component could any mutation in PTEN be demonstrated.

One tumour (CS 5) had two mutations in the PTEN gene, both significant. Both mutations were frameshift mutations, one in exon 7 and one in exon 8. Both these mutations in themselves will produce a truncated non-functional protein product and are therefore considered significant and disease causing. It is not known whether these mutations involved both alleles. This mutational pattern can be a cause for microsatellite instability as discussed earlier in this chapter (1.2.7). Indeed this tumour, CS 5, did demonstrate MSI as discussed above.

One missense mutation was found in tumour CS 15 (G-to-A in codon 130) and one missense mutation in tumour CS 19 (G-to-A in codon 15). The latter is a unique mutation not previously reported. Both of these mutations are thought to be disease causing.

Both of these tumours had endometroid epithelial component. Neither of these tumours displayed MSI or LOH in any of the chosen genetic tests.

Table 3.20: Mutations in the PTEN gene in carcinosarcomas.

Tumour	Mutation type	Exon	Nucleotide change	Effect
CS 5	frameshift	7	c.800delA	Stop at 275
CS 5	frameshift	8	c.968delA	Stop at 343
CS 15	missense	5	c.389G to A	Arg130Gln
CS 19	missense	1	c.44G to A	Arg15Lys

4.4.4   Endometrial str...

No mutations were found in any of the six endometrial stromal sarcomas included in this study or in the normal myometrium

representing normal DNA. We could not demonstrate any involvement of the PTEN gene in the tumorigenesis of this very uncommon mesenchymal tumour. A significant role for PTEN in this tumour type is not suspected.

4.4.5   Polymorphisms and pten-protein aberration

We did not find any polymorphisms in the PTEN gene. In this study only PTEN gene mutations were addressed. We did not investigate the occurrence of pten-protein aberrations, protein expression as measured by semi-quantitative immunohistochemistry or pten-protein function.

Influence of the gene mutations on protein length is a predictable event influencing protein function in a mutation specific way.

4.5 Correlation between clinical findings and molecular results

No PTEN mutations were found in any leiomyoma.

The one leiomyosarcoma that had a mutated copy of the PTEN gene had no differentiating clinical characteristics.

One of four Caucasian patients with CS had a PTEN mutation, while two of nineteen African patients with the same tumour were shown to have a PTEN mutation. Due to the small numbers, this difference cannot be considered significant. However, studies on endometroid endometrial carcinoma have indicated a much lower involvement of the PTEN gene in African women than in Caucasians. The findings of our own study of endometroid adenocarcinoma have been discussed in chapter 2 and displayed in table 2.9.

Some authors (Maxwell et al 1996) found an incidence as low as 5% for PTEN mutations in African-American women with endometrial cancer, suggesting a very small role for the PTEN gene in African women. This racial disparity described in endometrial cancer would be expected to persist into the product of genetic progression to a phenotypically divers tumour, namely the carcinosarcoma with endometroid epithelial component.

No PTEN mutations were found in any of the six endometrial stromal sarcomas.

Due to the small number of mutations, no attempt was made to correlate these findings with other clinical findings, like age or stage.

4.6 Correlation between histology findings and molecular results

Due to the small sample size and the low incidence of mutations, it was not statistically reliable to correlate the pathological sarcoma types with the presence or absence of PTEN mutations. Our observations and subjective interpretation are described.

In this dataset, the incidence of PTEN mutations ranged from none (all endometrial stromal sarcomas) to one in 19 or ~6% (all leiomyosarcomas) and three in 23 or ~14% (all carcinosarcomas). The highest incidence was three in eighteen or 17% in all CS tumours with an endometroid carcinoma element.

Although the numbers are too small for any statistical analysis, these findings possibly represent a correlation between the genotype and the phenotype of the tumour. It would be logical that the genetic aberrations found in tumour cells would also determine the phenotypic and histologic appearance of the tumour (Fujii et al 2000).

5.   Interpretation and discussion

5.1 Benign myometrium

No PTEN mutations were found in any of the tissue samples of benign germline tissue. This is what was expected as uterine sarcomas and leiomyomas have never been implicated as tumour types that occur in patients with the PTEN associated syndromes.

In our study these samples also served as controls for the method and to confirm that any mutations found are indeed somatic.

5.2 Leiomyomas, leiomyosarcomas and endometrial stromal sarcomas

Like in other mesenchymal tumours, we did not find important involvement of the PTEN gene in any of the uterine mesenchymal tumours. We could not detect any PTEN mutations in any of the endometrial stromal sarcomas or in any of the uterine leiomyomas. We detected only one mutation in the nineteen leiomyosarcomas examined. In this rare case, PTEN mutation seems to be involved in the lack of growth control and thus somewhere in the change from normal cell to a cell with neoplastic behaviour.

5.3 Carcinosarcomas

Carcinosarcomas differ from the other uterine sarcomas. These tumours probably originate from the epithelial cells and are thus rather carcinomas with de-differentiation. The findings of this study confirm that these tumours differ also on molecular level from the other sarcomas and from the benign mesenchymal tumours. Indeed CS seems to have more in common on molecular level with endometrial carcinoma than with LMS.

All carcinosarcomas are poorly differentiated tumours and as such similar results were expected as in poorly differentiated endometrial carcinoma. We found PTEN mutations in 3 of 23 tumours (13%). In our own dataset of endometrial carcinoma (chapter 2), three of ten grade 3 endometrial carcinomas (30%) displayed PTEN mutations, which seem a bit higher than in the sarcoma group. Non-mutational involvement of this tumour suppressor gene is expected in many more tumours.

Considering only those with endometroid epithelium, we found around a 17% incidence of PTEN mutations in CS with endometroid epithelium. In poorly differentiated endometroid carcinoma, we found a 50% incidence in the small subset of six patients, with identical incidences in both population groups. Our findings in endometroid carcinosarcomas correlates well with that reported in poorly differentiated endometroid adenocarcinoma of the endometrium by Maxwell et al (2000).

The incidence of PTEN mutation in African women with uterine sarcoma with an endometroid carcinoma component was ~12% (two of 17 tumours), while the incidence in Caucasians was ~30% (one of three tumours). Although in keeping with previous suggestions of racial disparity, our sample size was far too small to confirm that this trend extends to the sarcomas. In addition our findings in endometrial carcinoma did not support disparity between population groups.

The finding of two disease causing and protein truncation mutations in one tumour (CS 5) deserves mentioning. This finding in itself is highly significant and proves that PTEN somatic mutations can be involved in the tumorigenesis of carcinosarcomas. It also demonstrates that the inactivity of this protein can be associated with aggressive tumour growth (without normal inhibition) and aggressive tumour types in the uterus. Previous reports often reported an association with well differentiated types and good prognosis in the uterus while in other tissue types the opposite may be true (eg. brain tumours).

The association of these two mutations with the genetic finding of MSI is also of significance. Indeed it is believed that this severe inactivation of the pten protein will be the direct cause of the MSI, which is not usually associated with a single disease causing PTEN mutation.

Due to the small numbers assessed and the small number of mutations, it was not considered accurate to try to relate the presence or absence of mutations to other clinical and pathological variables.

5.4 Strenghts, limitations and recommendations

This study was one of the first in the world to study sarcoma and uterine sarcoma. It is definitely the first South African study and the only one to include both Caucasian and Black patients. It was also the first study of PTEN involvement in uterine leiomyoma.

Similar to the study of endometrial carcinoma, this study was limited to mutation analysis. No attempt was made to study pten protein levels or activity. It would be interesting to correlate gene mutations to protein expression and activity.

The findings in CS provide further strong support for the hypothesis that the pathogenesis of CS and endometrial carcinomas overlaps. Carcinosarcoma seems to be the least differentiated of all uterine carcinomas. On the other hand the different uterine sarcomas share very little in terms of etiology. It will be interesting to compare more molecular findings between the different uterine neoplasms.

Carcinosarcoma of the uterus is the first non-endometrioid, and the first non-epithelial gynaecological cancer where PTEN-mutations are found in such a high percentage of cases. To our knowledge this is also the first sarcoma where PTEN is found to play an important role.

The findings of this study shows important involvement of this tumour suppressor gene in the development of carcinosarcomas of the uterus. We could not, however, demonstrate when in the carcinogenetic pathway (early or late) these mutations occur. It would be hugely interesting to answer this question, but a suitable scientific model of study is outstanding. The most probable pre-cursor lesion for carcinosarcoma is atypical endometrial hyperplasia or early endometrial carcinoma.

The significance of all the interesting findings of this study is limited by small numbers. Unfortunately sarcomas are rare neoplasms and the methods used for mutation analysis are tedious. It would be useful to compare our findings to similar studies in future to complete the picture of PTEN involvement.