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Review Article

A potpourri of pathogenetic pathways in endometrial carcinoma with a focus on Lynch Syndrome

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ABSTRACT

Endometrial carcinoma is the most frequently occurring female genital tract malignancy in developed nations, with a rising annual incidence. Endometrioid endometrial carcinoma (EEC), the most common histological variant, differs in morphologic and molecular characteristics from serous carcinomas but morphological distinction of high-grade EECs from serous carcinomas may prove difficult. Thus, molecular categorization of tumors may allow for better tumor classification with greater insight into the underlying biology of endometrial carcinomas with new therapeutic options.

Microsatellite instability (MSI) is a commonly occurring molecular aberration in EECs and has been identified in most Lynch Syndrome (LS) associated tumors. This tumor syndrome predisposes afflicted individuals to a myriad of tumors including endometrial carcinoma. Herein, the molecular signature of endometrial tumors as well as LS, and its clinical manifestations are reviewed. Understanding of the pathogenetic pathways allows for greater comprehension of occurrences at a molecular level which are then appreciated at a cellular and tissue level, by the histopathologist.

The molecular classification of endometrial tumors allows for further targeted therapeutic options for affected patients. Screening tests for patients with suspected LS enables surveillance of other tumors in the affected patient and her family with the potential to decrease morbidity and mortality. It is envisioned that this overview will allow for enhanced comprehension of genetic pathways by practicing pathologists, oncologists, gynecologists and other members of the multidisciplinary team, all of whom are involved in the management of the patient with an endometrial malignancy.

1. Background

Endometrial carcinoma is the most frequently occurring female genital tract malignancy in developed nations; and in western societies, is one of the most common malignancies in females [1-4]. Endometrial carcinoma is anticipated to affect approximately 7% of females in the United States of America alone, in 2018. [1-4] Worldwide, endometrial carcinoma accounts for roughly, 4% of all tumors [4]. This article will delve into the molecular aberrations of endometrial tumors and will highlight the recent advances in genetic profiling of these neoplasms. Furthermore, this article discusses microsatellite instability, which has been identified in approximately 90% of endometrial carcinomas associated with LS [5] and provides the practising histopathologist with an in-depth understanding of the underlying fundamental mechanisms that culminate in DNA mismatch repair, which have also been documented in up to 30% of sporadic endometrial carcinomas [2]. This will facilitate a more thorough comprehension of the basis of the hereditary tumor predisposition syndrome, known as Lynch Syndrome (LS). It must be borne in mind that it is a mutation of MLH1, MSH2, MSH6 or PMS2 genes that defines LS and not the absence of staining of one the immunohistochemical stains. Lynch Syndrome and its clinical correlates are reviewed in detail.

There is currently a rising incidence of endometrial carcinoma in the western world which has been ascribed to an increased usage of hormonal therapy such as Tamoxifen for breast carcinoma, the associated occurrence of obesity, as well as a prolonged life span [6-10]. Tamoxifen has been shown to possess anti-estrogenic effects in breast parenchyma but in endometrial tissue, Tamoxifen provides an estrogen rich environment [9]. This may then set the scene for endometrial hyperplasia and possible neoplastic transformation. Another source of

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exogenous estrogen is hormonal therapy ingested in the absence of progesterone [9]. Unopposed estrogen allows for proliferation of the endometrial lining without the counter-estrogenic effects of progesterone. Increased adiposity in obese females serves as a reservoir for endogenous estrogen [9]. There is currently a rising percentage of overweight and obese females seen in developing countries as well as in developed nations. This may therefore herald an increase in estrogen associated neoplasms such as endometrial carcinoma [11]. Ovarian abnormalities such as cases of polycystic ovarian syndrome or some ovarian neoplasms may serve as additional alternate sources of endogenous estrogen [9].

In 1983, Bokhman proposed the dualistic clinicopathologic model of endometrial carcinoma, which divided endometrial tumors into those that were estrogen dependent (Type I) and those that were estrogen-independent (Type II tumors) [2,6,10,12-14] This system, whilst rigid, does allow for an understanding of the pathogenetic pathways of endometrial tumors [15]. There are many instances in which there is blurring of the morphological pictures of the two tumor groups which makes classification into either group difficult. Furthermore, use of this classification model for patient prognostication is inadequate as tumor relapses may occur in up to one fifth of Type I tumors whilst up to half of Type II neoplasms do not relapse [10]. Thus, this model is not a suitable tool for assessing patient risk and it is not used for tumor staging, but does facilitate conceptualisation of the pathogenesis of endometrial carcinomas [15]. Type I tumors or Endometrioid endometrial carcinomas, (EEC) the most common histological variant, have a propensity to arise in peri-menopausal or premenopausal females [6]. EECs are usually low grade carcinomas often arising in the setting of endometrial hyperplasia and in an estrogen rich environment. These tumors bear histological resemblance to normal endometrium (Fig. 1). [2,6,13,14] The spread of these neoplasms is usually limited to the uterus [14]. Such tumors have a reasonably good clinical outcome and the majority of patients are often cured following a hysterectomy [2,13,14]. The Type II tumors, or non-Endometrioid Endometrial Carcinomas (non-EEC) include serous and clear cell carcinomas and carcinosarcomas which have a relatively worse outcome than Type I neoplasms [2,13,14,16] This group of tumors is estrogen independent and has a proclivity to arise in older females who are usually not obese. [2,17] Treatment modalities for EECs differ to those used for non-EECs. Patients with early-stage EECs may be cured of disease by surgery whilst others may require adjuvant radiotherapy; whereas patients with non-EECs undergo radiation and/or chemotherapy which is the treatment offered to high-staged tumors of both histological subtypes [15,17]. As such, it may be seen that accurate subtyping of tumors is required for appropriate, judicious adjuvant therapy [17,18]. Furthermore, studies have pointed toward poor reproducibility of histological subtypes especially with regard to high grade tumors [15]. Thus, molecular categorization of tumors may allow for a more accurate tumor classification [17].

The current risk stratification systems for endometrial carcinomas all separate patients into various tiers following surgical staging [15]. A patient's risk of recurrent disease is assessed on clinical and pathological factors such as age, histological tumor subtype, grade of tumor, lymphovascular involvement and stage of disease [19]. Patients are classified into one of three risk groups. The postoperative radiation therapy for endometrial carcinoma (PORTEC) 1 and 2 clinical trials have demonstrated that patients that fall into the high to intermediate risk category benefit from vaginal brachytherapy whilst patients in the low risk group do not need to be subjected to adjuvant therapy. Nevertheless, many patients are still under or overtreated [20]. Thus, there is a need for early assessment of the biological potential of each patient's tumor so that personalised treatment options may be applied [15].

Molecular studies undertaken by The Cancer Genome Atlas (TCGA) Research Network in 2013 classified endometrial tumors into four groups instead of the traditional, rigid division into Type I and II endometrial carcinomas [17]. The Cancer Genome Atlas Research Network utilized microsatellite assays, whole genome sequencing, analysis of copy number as well as exome sequencing on endometrial and serous carcinomas [15,17]. The four groups comprise “microsatellite instability hypermutated, DNA polymerase epsilon or *POLE* ultramutated, copy-number high and copy-number low” [17]. Such classification may provide invaluable information on tumor biology with astute resultant treatment options for patients. This group of investigators found that 7% of endometrial tumors tested showed a nucleotide change of Cytosine➔Adenosine and these tumors had an unusually high rate of mutations in the exonuclease domain of *POLE*. *POLE* encodes a catalytic and proofreading component of DNA polymerase epsilon, which functions to initiate DNA strand replication. *POLE*'s precise inclusion of bases together with the exonuclease proofreading role results in few mutations in the daughter strand. It was shown that DNA polymerase substitutions resulted in suppression or inactivation of the proof-reading functions which then served to increase errors in replication with the resultant hypermutated phenotype [15,17] The *POLE* group of tumors showed an endometrioid morphology and had mutations in the following genes: PTEN, PIK3CA, ARID1A, FBXW7, PIK3R1 and KRAS. These neoplasms showed an endometrioid morphology and had a better overall prognosis, even in high-grade tumors [15,17,21]. The microsatellite instability (MSI) hypermutated group had an endometrioid morphology and showed mutations in PTEN, PIK3CA, KRAS, ARID1A, RPL22 and PIK3R1. Mutations occurred at a far greater frequency than those of microsatellite stable tumors [17,21]. It has been suggested that the high rate of mutation results in the formation of new antigens which leads to stimulation of the immune system and ultimately culminates in heightened anti-neoplastic activity by the body's defences [22]. The copy number high group was identified by means of microarrays in which recurring regions of deletions or amplifications were recognized. This group of tumors showed mutations in TP53, PIK3CA, PPP2R1A as well as chromosomal instability. Most of these tumors demonstrated serous morphology, whilst a quarter of these tumors had grade 3 endometrioid features. The copy number low group encompassed all those cases that did not fall into the other three groups, namely copy number high, MSI hypermutated or *POLE* ultramutated [15,17]. These tumors showed a low rate of mutation with grade 1 or 2 endometrioid features [17,21]. This study demonstrated a better correlation of the molecular classification than the histopathological findings, with regard to the clinical outcome [22]. Whilst such classification provides immense information into the underlying biology of endometrial carcinomas with possible therapeutic options, molecular testing such as POLE polymerase chain reaction (PCR) or sequencing is not routinely available to all practicing histopathologists, especially not in the developing world; and requires specialised laboratory personnel to undertake such tests. Studies have subsequently been performed in an attempt to replicate the survival curves and molecular stratification obtained by the TCGA using formalin-fixed paraffin embedded tissue. [15,20,23] The Leiden classifier by Stelloo and colleagues [19] used sequencing (either Sanger or next-generation) of hotspots in exons 9 to 14 of the exonuclease domain to identify POLE mutations. p53 Mutant tumors were identified by immunohistochemistry (IHC) as well as by mutational testing and characterised the copy number high group of tumors. Microsatellite instability was assessed by PCR or the mismatch repair (MMR) immunohistochemical stains. The fourth group was those that did not fit into the other three groups and were termed “no significant molecular profile (NSMP)” [19]. A number of other genes such as PTEN, BRAF, CTNNB1 and FGFR2 amongst others, were tested for mutations. In cases where there was insufficient tissue to perform all the tests, the cases were excluded. In addition, tumors which had more than one molecular abnormality were not classified. Based on these exclusions, the order of testing did not matter [15,19]. Stelloo *et al* [19] evaluated endometrial carcinomas that were of high recurrence risk, from the PORTEC-3 trial and found that the POLE and MSI groups had better

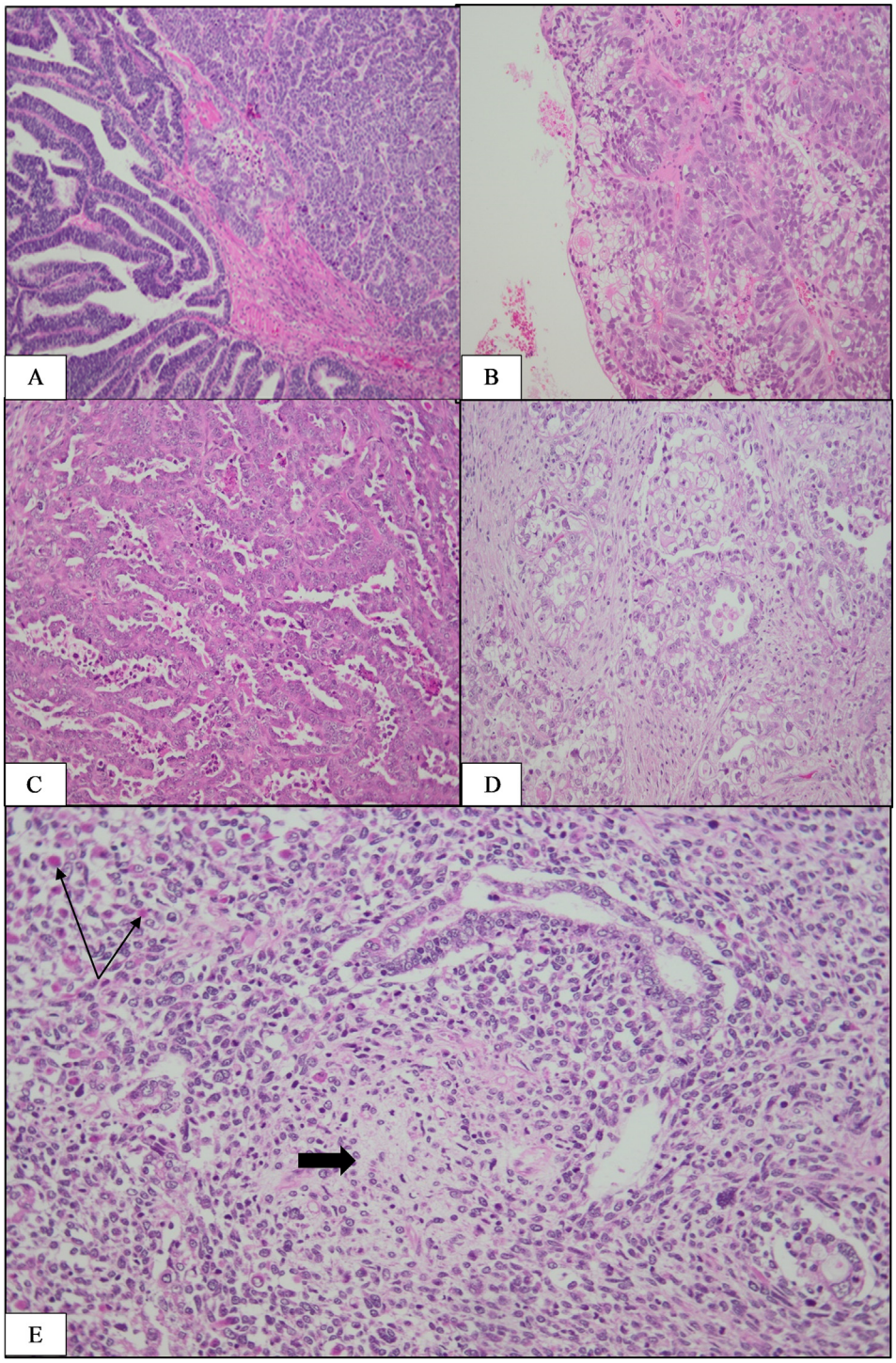

Fig. 1. A composite image of endometrial carcinomas. 1A shows endometrioid endometrial carcinoma. 1B shows a mucinous endometrial carcinoma. 1C demonstrates a serous carcinoma in which hobnailed cells are seen. 1D shows a clear cell carcinoma with tubules lined by variably pleomorphic cells. 1E illustrates a carcinosarcoma with rhabdomyoblasts identified at the arrows and a lobule of cartilage seen at the arrow head. 2 μm H&E stained section. Original magnification: 200×.

outcomes than the p53 and low copy number/NSMP groups. In addition, tumors that were microsatellite stable and were wild-type for testing of exon 3 of CTNNB1 had a favourable prognosis whereas tumors that demonstrated microsatellite instability or mutations on exon 3 of CTNNB1 had an intermediate prognosis [19] The PROmISE (Proactive molecular risk classifier for endometrial cancer) model provides a step-wise algorithm of testing to be performed with identification of the different molecular subgroups. This algorithm suggests that new endometrial biopsies undergo MMR testing by IHC for PMS2 and MSH6. Those that have one or more negative stains are then regarded as being MMR-deficient, whilst those with retention of staining should undergo POLE testing. If MMR testing is missing, then the tumor is placed in the unclassifiable group. Tumors undergoing POLE testing are then assessed for mutations or are considered POLE wild-type. If POLE testing is missing, then the tumor is placed in the unclassifiable group. Wild-type POLE tumors may then undergo p53 IHC testing

which may categorise tumors as either p53 mutated (either a missense mutation or null-mutation) or p53 wild-type. For tumors in which p53 is missing, the tumor is unclassified. This algorithm facilitates categorization of endometrial tumors into one of the four groups and had replicated results of other studies whereby, for example, POLE mutated groups had a better prognosis despite the tumors being histologically high grade, showing lymphovascular invasion and being deeply infiltrating into myometrium [15,24]. This stratification of tumors in the preoperative setting may assist clinicians decide whether or not a lymph node dissection is required, whereas in the postoperative setting it may point toward whether or not adjuvant treatment is needed [18]. There is currently an increase in guidelines advocating reflex screening test for LS on endometrial biopsy specimens in the preoperative setting, so it is plausible that testing of POLE and p53 would be included in the armamentarium of preoperative tests. Tumors that have POLE mutations rarely have spread beyond the uterus and as such, total hysterectomy and bilateral salpingo-oophorectomy would suffice in the management of these neoplasms. The identification of the specific mutant MMR in endometrial carcinoma may have clinical implications on definitive management such as in cases of MSH6 mutations in which there is a later age of onset, as well as a reduced lifetime risk of tumors. A mutation in MSH6 may result in a delay in tumor-preventative surgery as opposed to a mutation in one of the other MMR genes associated with LS [22].

Patients with p53 mutated tumors however, would require lymph node dissection and omentectomy at the time of hysterectomy. Regarding postoperative adjuvant treatment for high to intermediate risk patients, the current Postoperative radiation therapy for endometrial carcinoma or PORTEC-4a trial, which continues to 2025, is a clinical endometrial carcinoma trial in which molecular aberrations are used to investigate efficacy of differential adjuvant treatments [25]. Differential adjuvant treatments constructed to molecular abnormalities will be compared to use of vaginal brachytherapy which is the current standard of care. Such trials may provide evidence for use of targeted treatment in the future [18,25].

Whilst immunohistochemistry may be available to most pathologists, it is important to remember that not all centres in the United States of America and Europe, and especially not those in developing countries, have access to PCR or sequencing technologies. This then implies that such stratification of endometrial carcinomas would not be possible globally. At present, according to unpublished data (as cited by Bosse *et al* [23]), the POLE immunohistochemical antibody is not suitable for diagnostic use [23]. It is anticipated that in due course, a stain for clinical application will be commercially available. Needless to say, this may prove invaluable and may be used to classify endometrial tumors preoperatively with the aim of optimal patient treatment.

2. Introduction

At a molecular level, Type I tumors are associated with microsatellite instability in addition to mutations in *PTEN, PIK3CA, KRAS* and CTNNB1. [2,14-17,22,26-31] In contradistinction, most Type II tumors harbour p53 mutations or Her2/neu amplifications [14]. These tumors may also show loss of heterozygosity (LOH) on various chromosomes [14,15,17,22,26-31]. Type I tumors may however have p53 mutations and similarly, Type II tumors may have mutations such as PIK3CA; which are usually associated with Type I neoplasms.

3. Molecular pathways in endometrioid endometrial carcinomas

3.1. Microsatellite instability

MSI is one of the most commonly occurring molecular aberrations in Type I tumors and has been identified in most tumors associated with Lynch syndrome in addition to up to 30% of sporadically occurring tumors [16,17,27,28,32]. Microsatellite instability is found predominantly in endometrioid endometrial carcinomas [31]. A small proportion of NEECs may be associated with LS, but this is far less common than that seen in EECs [33]. A study undertaken by Broaddus *et al* [33] illustrated that in their cohort, all NEECs associated with LS harboured MSH2 mutations and that less NEECs were identified in LS patients than compared to the general population [33].

Microsatellites are one to five base pair sequences on DNA that are repeated a number of times and it is these short-tandem repeats that are prone to alterations in mismatch repair [16,29,30]. A repeat of CA is the most commonly occurring microsatellite identified in humans [16]. In general, errors in replication tend to be corrected by the “DNA mismatch repair system” [34-38] This system repairs “base-base mismatches” as well as “insertion-deletion loops” found in microsatellite regions [36]. Microsatellites may occur in coding and non-coding DNA sequences, for example; centromeres and telomeres [17,34]. The gamut of mutational types is vast, with mutations being deleterious or silent [36]. Morphologically, deleterious mutations result in an absence of immunohistochemical staining of tumor cells [36]. Insertion-deletion loops that are not rectified, may cause frameshift mutations [36]. Coding sequences containing missense mutations are involved in carcinogenesis, as seen in EECs [17,34,36]. Between 25 and 30% of non-heritable, sporadically occurring endometrial carcinomas demonstrate microsatellite instability [27,28].

The MMR system is found in both eukaryotes and prokaryotes [36]. Mutated eukaryotic genes are referred to as “Mut”. MutS homologs include MSH2, MSH6 and MSH3. MutL homologs include MLH1, PMS1 and PMS2 (Post-Meiotic Segregation proteins) [36,37]. The letter “h” is the prefix assigned to the genes and proteins in human *e.g.* hMSH2 [36]. There are currently seven MMR genes documented in humans, namely MLH1, MLH3, MSH2, MSH3, MSH6, PMS1 and PMS2 [39]. MutH homologs have not been identified in humans [39]. There are major and minor MMR genes which function as an interconnected network whereby the major genes result in protein expression and the minor genes function to stabilise the major genes [37,38]. Major genes comprise MLH1 and MSH2 whilst minor genes consist of PMS2, MSH6 and MSH3 as well as others.

MHS2 and MSH6 together form the “MutSα” complex which is capable of recognizing mismatches in base pairs and is involved in repair of single base pair and insertion-deletion mutations and may also cause apoptosis. [39,40] MutSβ complex is composed of MSH2 and MSH3 which recognises and mends short deletions or insertions [36,39]. Both MutSα and MutSβ complexes require MSH2 for their functioning. [39] MLH1 and PMS2 form the MutLα heterodimer which combines with MutS complexes to remove and resynthesize anomalous DNA [36]. Mismatch repair cannot occur if any of the four proteins, namely MLH1, PMS2, MHS2 and MSH6 are functionally impaired [39].

3.2. MLH1 promoter hypermethylation

DNA methylation is a form of epigenetic processes that also includes alteration of histones, remodelling of chromatin together with microRNAs [41]. Methylation is the process of addition of a methyl group to cytosine nucleotides in CpG islands in the MLH1 gene promoter region [42]. It is these methyl groups attached to cytosine nucleotides that extend into the major groove of DNA strands and thus prevent DNA transcription by inhibiting transcription factors from annealing to DNA [42]. Furthermore, methyl groups draw proteins that bind methyl to them, with resultant chromatin compaction and eventual gene silencing [42].

Up to 75% of MSI in EECs have been identified as sporadic phenomena that arise following hypermethylation with subsequent inactivation of the MLH1 promoter region [43]. MMR IHC testing together with MSI by PCR and hypermethylation studies can suggest which patients may have LS. Negative staining of MLH1 or PMS2 on immunohistochemistry together with MSI-H by PCR and a negative result for MLH1 promoter hypermethylation point toward the patient

having LS [43]. However, negative staining of MLH1 or PMS2 on immunohistochemistry, MSI-H by PCR and a positive result for MLH1 hypermethylation studies suggest that the patient does not have LS and this is a sporadic occurrence [43].

3.3. The PTEN pathway

PTEN is a tumor suppressor gene located on chromosome 10q23.3 that encodes the enzyme phosphatidylinositol phosphatase which dephosphorylates phosphatidylinositol-trisphosphate (PIP3). PTEN's mechanism of action is to antagonise the phosphatidylinositol 3-kinase and Protein Kinase B (PI3K/AKT) pathway [14,44]. Thus PTEN dephosphorylates PIP3, which is a product of PI3K [14,44]. Lowered PTEN activity results in greater cell proliferation as well as increased cell survival by means of altering pathways of signal transduction [14,44]. Inactivation of PTEN may result from varying mechanisms such as loss of heterozygosity on chromosome 10q23, promoter hypermethylation or mutation [44].

Studies undertaken by Salvesen *et al* [45] and Risinger *et al* [46] showed that endometrial tumors containing PTEN mutations had an endometrioid morphology, had a lower histological grade and stage and thus had a better overall outcome. However, studies undertaken more recently have suggested that PTEN promoter hypermethylation is associated with a higher stage of disease and that only mutations outside exons 5 to 7, suggest a better outcome [14]. Between 60 and 86% of microsatellite unstable endometrioid endometrial carcinomas have PTEN mutations [27,44]. Prat *et al* [44] identified two short coding mononuclear repeats in 44% of microsatellite unstable endometrial carcinomas which refers to mismatch repair deficiencies resulting in PTEN mutations [44].

3.4. The PI3K pathway

As alluded to above, function of the Phosphatidylinositol 3-kinase (PI3K) pathway is coupled to PTEN functionality. The PI3K-AKT pathway is involved in cellular growth, proliferation and cell survival [26]. PI3K is a heterodimeric enzyme that has a regulatory subunit (p85) as well as a catalytic subunit (p110) [27,31,44]. The PI3K gene is situated on chromosome 3q26.32 and codes for the p110α catalytic subunit PIK3CA [27,31,44]. Amplification of this region results in enhanced PIK3CA activity [44]. PIK3CA mutation or increased activation is identified in many neoplasms [14,44]. PIK3CA mutations are oncogenic and have been identified in up to 36% of endometrial carcinomas. [44] Concomitant mutations in PIK3CA and PTEN have been documented in up to a quarter of endometrial carcinomas [31,44]. PIK3CA mutations in exon 20 have been associated with poor histological prognostic factors such as myometrial infiltration, lymphovascular invasion and high-grade morphology [3,31].

Binding of PI3K to a growth factor receptor kinase results in the formation of PIP3 which then results in activation of other pathways which direct cell growth, proliferation, motility, adhesion, survival and apoptosis [44]. Thus it may be seen that such regulation promotes cellular growth and proliferation with simultaneous inhibition of apoptosis [44]. Mammalian target of rapamycin or mTor, is downstream in the PI3K-PTEN-AKT pathway and becomes activated in cases of PI3K or PTEN mutations. mTor inhibitor pharmacological agents have a role to play in tumors which have PTEN inactivation such as endometrial carcinoma [28].

PIK3CA mutations have not displayed any correlation with either MSI or CTNNB1. Furthermore, mutual exclusivity between PIK3CA mutations and K-RAS mutations has been identified [26].

3.5. The RAS-RAF-MEK-ERK pathway

3.5.1. RAS

The RAS-RAF-MEK-ERK signalling pathway is often activated and has an important role in several tumors, including endometrioid and mucinous endometrial carcinomas [14,26,31]. RAS proteins facilitate signal transduction between cell surface and nucleus through the PI3K-PTEN-AKT and RAS-RAF-MEK-ERK pathways [14,26]. Therefore, RAS proteins are involved in the regulation of various cell activities such as cell proliferation and survival [26]. The RAS family of genes comprises the following: H, K and N-RAS which encode for proteins p21that are present on the inner cell membrane and GTPase activity [47]. Substitutions of single amino acids at codons 12, 13 and 61 have been identified as causes for activating mutations due to loss of GTPase activity [44,48]. KRAS, or Kirsten rat sarcoma viral oncogene homologue, may bind directly to PIK3CA and upregulate that pathway as well as result in activation of the AKT pathway with resultant inhibition of apoptosis [14,28,48]. Mutations in KRAS have been identified in between 10 and 30% of endometrial carcinoma and have been observed in atypical hyperplasia and are thus considered an early event in tumorigenesis of endometrial carcinoma [26,27,31,44,47].

Several researchers have demonstrated an increase in KRAS mutations in cases of endometrial carcinoma that have microsatellite instability [15,20,49,50]. Some authors have suggested that MSI and KRAS mutations are related to one another and occur concomitantly.

There has been no relationship demonstrated between KRAS mutations and age of patient, extent of myometrial invasion, histological grade, stage of tumor or overall clinical outcome [49,50].

3.5.2. RASSF1A

RASSF1A is the KRAS effector which is an important tumor suppressor that plays a role in arrest of the cell cycle, preservation of genomic stability, and control of apoptosis [15]. It has an inhibitory effect on growth signals and there is loss of this inhibitory function during carcinogenesis [25]. Thus loss of RASSF1A function results in increased signalling of the pathway [15]. Loss of RASSF1A function has been attributed to promoter hypermethylation as observed in numerous tumors including endometrial carcinomas [15,25]. Hypermethylation of RASSF1A has been shown to correlate with an advanced stage of disease and has been shown to occur more commonly in tumors that are microsatellite unstable. As such, it has thus been hypothesized that there is a methylator phenotype that affects the MLH1 gene in addition to other genes such as RASSFIA. Hypermethylation of RASSF1A is more likely to be seen in tumors that do not have KRAS mutations [26].

3.5.3. BRAF

BRAF is a member of the RAS-RAF-MEK-ERK pathway. BRAF mutations are mutually exclusive with hypermethylation of RASSF1A and KRAS mutations [26]. In addition to MLH1 promoter hypermethylation, mutations in *BRAF* V600E are in support of sporadic occurrence of a colorectal tumor [51]. The occurrence of BRAF mutations have a significant difference in colorectal carcinomas and endometrial carcinomas; in the latter, BRAF is mutated only extremely rarely in studies from the west and is thus not considered an indication of epigenetic loss of MLH1 [26]. Feng *et al* [52] identified BRAF mutations in 21% of their cases of endometrial carcinoma. Many cases that were negative for MLH1 by immunohistochemistry were BRAF mutated which suggests that they may be associated with microsatellite instability [52]. It has been suggested that the high incidence of BRAF mutations noted by Feng *et al* [52] may be attributed to different ethnic populations studied. This however has not been fully established [26].

3.6. The WNT pathway

3.6.1. CTNNB1 - β-catenin

The CTNNB1 gene is located on chromosome 3p21 and encodes the protein, β-Catenin [14,26,27,44]. The WNT(Wingless-type) family encodes glycoproteins that facilitate growth and differentiation [53]. In the WNT pathway, Frizzled (FZD) receptors facilitate signal transduction *via* intracellular proteins to CTNNB1, the transcriptional regulator

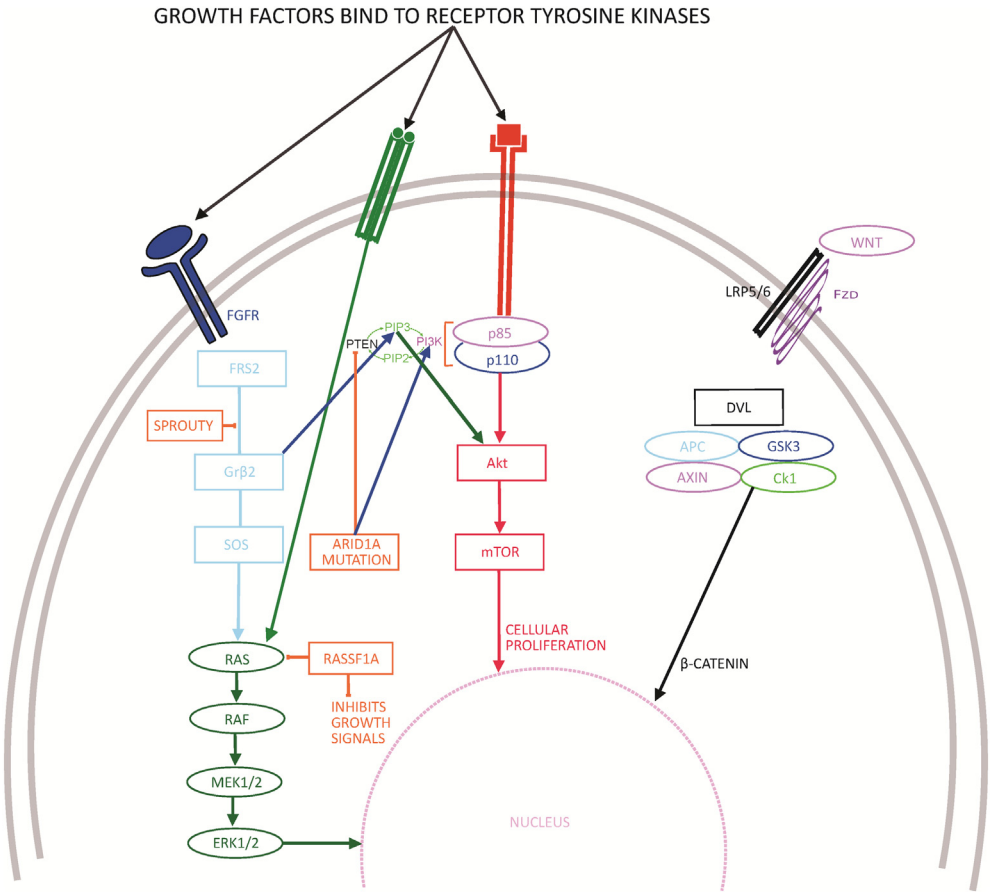

Fig. 2. Illustrates the pathogenetic pathways in endometrioid endometrial carcinoma.

[14]. β-Catenin forms part of the E-Cadherin-Catenin complex and is an adherens junction protein which ensures adhesion between cells. [26,27,44] Free β-Catenin within the cytoplasm of cells is able to come into contact with the Adenomatous Polyposis Coli (APC) protein and may facilitate transcription. APC proteins, together with Glycogen Synthase Kinase 3β (GSK3β) cause serine-threonine residues coded in exon 3 of CTNNB1, to undergo phosphorylation culminating in the degradation of β-Catenin *via* the ubiquitin-proteosome pathway. Thus APC acts to down-regulate β-Catenin. [14,31,44] When WNT binds to FZD and lipoprotein receptor-related protein 5 or 6 (LRP5/6) they form a complex that acts together with Dishevelled (DVL) and Axin causing β-Catenin stabilisation by inhibiting β-Catenin phosphorylation [54]. This then facilitates cellular proliferation [54]. Mutations in exon 3 of the CTNNB1 gene causes protein stabilisation, accumulation in the cytoplasm and nucleus, facilitation of signal transduction together with transcription of genes that are implicated in carcinogenesis [14,31,44]. Thus abnormal build-up of β-Catenin arises from mutations in CTNNB1 as well as other genes which, *via* the LEF/Tcf pathway (T-cell factor/lymphoid enhancer factor), [53] results in transcription of genes such as CCND1 (cyclin D1) [14].

Between 14 and 44% of endometrial carcinomas have mutations in CTNNB1 [14,26,27,31,53]. However, mutations occurring within APC are not common in endometrial carcinomas in contrast to colorectal carcinomas [14,53]. Recent investigations by Eskander *et al* [53] have suggested a role for the WNT inhibitor, Dickkopf (Dkk) [53]. These authors demonstrated lowered levels of Dkk protein expression in endometrial carcinomas compared to control tissue. Decreased Dkk expression on an mRNA level correlated with higher histological grade, greater myometrial infiltration together with increased obesity [53].

β-Catenin mutations seem to exist separately from microsatellite instability, PTEN or KRAS mutations [31].

3.7. The fibroblast growth factor (FGF) pathway

Fibroblast growth factor is expressed in a multitude of tissue types and plays a vital role in regulating angiogenesis and wound repair in normal tissue and in neoplasias [55]. Activation of FGF receptors results in signal transduction through different pathways such as PI3K and RAS-RAF-MEK-ERK [55]. As in other pathways, regulation by various proteins exists in the FGF pathway. Notably, “Sprouty mammalian genes” or SPRY have a leading role. The SPRY proteins have been identified in controlling receptor tyrosine kinases in addition to the RAS pathway. Thus, SPRY proteins exert control over angiogenesis, cell differentiation and proliferation. FGF pathways may be affected in different manners such that FGF may behave as a tumor suppressor whilst in other cases it may take on the role of an oncogene with resultant cellular proliferation and migration [55].

There is evidence that in endometrial carcinomas SPRY2 is inactivated. Thus, the inhibitory effect on the FGF pathway is removed [55]. Mutations in the fibroblast growth factor receptor 2 (FGFR2) tyrosine kinase have been documented in between 6 and 12% of endometrioid endometrial carcinomas [26,27]. FGFR2 mutations have been found to be mutually exclusive with mutations in KRAS. In contradistinction, up to 77% of FGFR2 mutations and mutations in PTEN occur concomitantly [26,27,55]. FGFR2 is currently of great interest as this could be a potential pursuit for therapeutic modalities [28].

3.8. ARID1A

The AT-rich interaction domain (ARID) family is a tumor suppressor gene that encodes BAF250a which is a part of the SWI/SNF (mating type switching/sucrose non-fermenting) chromatin remodelling complex [26,56]. Abnormalities in ARID1A as well as BAF250a have been

identified in numerous endometrioid endometrial carcinomas. ARID1a mutations have been noted to occur in approximately 23% of microsatellite unstable endometrial carcinomas [17]. ARID1A results in inhibition of the PTEN pathway; but activates the PI3K and Akt-mTor pathway which then causes cellular proliferation [57]. ARID1 mutations have also been identified in undifferentiated carcinomas [58].

3.9. Epithelial to mesenchymal transitions (EMT) in undifferentiated carcinomas

Epithelial to mesenchymal transitions (EMT) are mechanisms by which epithelial cells develop a mesenchymal property and phenotype that allow for loss of intercellular adhesion and increased ease of cellular migration. This is facilitated by downregulation of E-cadherin and upregulation of known EMT associated proteins such as ZEB-1, SNAIL and TWIST due to upregulation of microRNA (miRNA) 200 series [58]. Undifferentiated carcinomas are a molecularly diverse group of tumors and have demonstrated mutations from the four molecular subgroups described by TCGA [58].

The pathogenetic pathways involved in endometrioid endometrial carcinogenesis are illustrated in a line diagram (Fig. 2).

4. Molecular pathways in non-endometrioid endometrial carcinomas

4.1. Serous carcinoma

4.1.1. TP53 – p53 mutations

TP53 is a tumor suppressor gene which is found on chromosome 17p13, that encodes for the nuclear p53 protein which is a transcription factor that in the presence of DNA damage, triggers expression of genes that cause arrest of the cell cycle with subsequent apoptosis [14,44]. Loss of p53 function results in the inhibition of apoptosis which causes loss of heterozygosity and aneuploidy with ensuing widespread genetic instability [14,44]. There is rapid breakdown of p53 under normal conditions and as such, there is no identification on an immunohistochemical level. Mutations of TP53 result in abnormal, non-functional p53 protein production which is not broken down and is therefore visible immunohistochemically [44]. It must be borne in mind that as a tumor suppressor, inactivation of both alleles either *via* mutation or through loss of heterozygosity is required. Hence, loss of heterozygosity of p53 may not necessarily culminate in protein overexpression. However, some p53 mutations have been described as causing loss of function with just a single allele being mutated, as a consequence of the p53 mutation having a negative effect on wild-type p53 [44]. Diffuse, strong positivity, or complete negativity on a p53 immunohistochemical stain is seen in serous carcinomas [32].

Studies have shown that up to 90% of serous carcinomas have TP53 mutations. Mutations of p53 occur early in carcinogenesis as evidenced by “p53 signatures” in which morphologically bland, benign appearing glands display strong p53 staining of their nuclei. P53 signatures are also identified in benign epithelium that lays contiguous to serous carcinoma [26]. Furthermore, p53 mutations have been observed in endometrial intraepithelial carcinoma (EIC) in which cytologically malignant cells are identified within the surface epithelial lining. Whilst EIC is confined to the endometrial epithelial lining or an endometrial polyp's surface, it has been associated with a higher stage and portends a poor prognosis [44].

Mutations of p53 are uncommon in endometrioid endometrial carcinomas, and have an incidence between 10 and 20%, most of which occur in grade 3 EECs [28,44]. Zero (0) percent of grade 1 EECs have had p53 mutations identified [26]. The presence of p53 mutations in EEC validates the belief that p53 is responsible for the evolution of EEC to non-endometrioid endometrial carcinoma whilst still maintaining the typical molecular features of EECs [44]. Overexpression of p53 has been correlated with both higher histological grade and tumor stage together with a worse overall prognosis [15,17,19].

4.1.2. HER2/neu – ERBB2

The oncogene HER2/neu or epidermal growth factor type II receptor encodes a tyrosine kinase. This serves as a receptor for growth factors and is involved in directing the ERBB pathway [14,26,44]. HER2/neu does not have its own ligand binding and as such, is not able to bind growth factors. However, HER2/neu heterodimerizes with HER1/erbB1, HER3/erbB3 and HER4/erbB4; all of which belong to the family of epidermal growth factor receptors (EGFR) [44]. Activation of HER2/neu culminates in greater cell proliferation by virtue of enhanced PI3K and Mitogen-activated protein kinase (MAPK) pathways [14,44]. Between 20 and 40% of endometrial carcinomas have been associated with HER2/neu overexpression. Furthermore, amplification of HER2/neu correlates with a higher histological grade, greater stage of disease and worse overall outcome [14].

4.1.3. CDKN2A (p16)

CDKN2A/p16 is a tumor suppressor that inhibits G1/S phase of the cell cycle. Studies have documented strong intensity, widespread staining of p16 in serous endometrial carcinomas whilst EEC have shown weak intensity, focal staining with p16 [26]. Positivity of p16 in serous carcinomas is thought to be related to loss of p53 function with resultant overexpression of p16 [59]. P16 may also be rendered inoperative by promoter hypermethylation, mutations and homozygous deletions [28,60].

4.1.4. E-cadherin

CDH1 is a tumor suppressor gene that codes for the adhesion molecule E-cadherin. Decreased expression or negativity of E-cadherin has been identified more often in serous and clear cell carcinomas of the endometrium than in endometrioid endometrial carcinomas. The precise molecular processes that result in decreased expression of E-cadherin are not entirely clear. Loss of heterozygosity of the CDH1 gene is a far more common occurrence in endometrial serous and clear cell carcinomas than EECs [26]. Loss of heterozygosity at chromosome 16q22.1 has been observed in approximately 60% of non-endometrioid endometrial carcinomas [27]. Whilst promoter hypermethylation of CDH1 is a common finding in endometrial carcinomas, it has not been found to always correlate with decreased expression of the E-cadherin protein [26]. Additional processes that may result in decreased E-cadherin expression include abnormalities in certain transcriptional repressors of E-cadherin. Dysregulation of these inhibitors cause their overexpression which ultimately results in lowered levels of E-cadherin. Loss of E-cadherin has been implicated in epithelial to mesenchymal transition, whereby cells lose their epithelial characteristics and adopt a mesenchymal phenotype [26]. Studies have revealed that high levels of E-cadherin are associated with a decrease in progression of disease and decreased disease associated mortality [26,44].

4.1.5. PPP2R1A

PP2A is coded for by the gene PPP2R1A, and is a serine-threonine phosphatase [26,61]. In its regular state, it serves to inactivate AKT, in the same manner that PTEN does [61]. Somatic mutations occur in up to 41% of serous endometrial carcinomas [26].

4.1.6. Cyclin E (CCNE)

Immunohistochemically, Cyclin E is overexpressed far more commonly in NEEC than in EECs. It is thought that the increased cyclin E may be attributed to amplification of the CCNE gene. Furthermore, there may be loss of function mutations in the tumor suppressor gene FBXW7 [26]. FBXW7 is dependent on p53 and encodes for a substrate recognition constituent of an ubiquitin-ligase composite. This complex is responsible for directing ubiquitin-mediated degradation of cyclin E. Studies have shown that mutations in FBWX7 and CCNE amplification result in increased levels of cyclin E protein. This then allows the cell

cycle to forge ahead with resultant cell proliferation and tumorigenesis [62].

4.1.7. Chromosomal instability

Chromosomal instability typifies non-endometrioid endometrial carcinomas. There are extensive chromosomal losses and gains all of which are indicative of aneuploidy. It has been shown that there is upregulation of genes that control the mitotic spindle checkpoint; such as STK15 which is a serine-threonine kinase that is needed for equal separation of chromosomes. STK15 overexpression results in raised numbers of centrosomes as well as aneuploidy [14]. There is often amplification of STK15 in NEECs [28,44].

4.1.8. PI3K pathway

This pathway has been described in Section 3 molecular pathways in EEC.

4.2. Clear cell carcinomas

Clear cell carcinomas were not included in the TCGA study and there is less known of this type of carcinoma in contrast to other endometrial tumors [63]. These tumors have mutations in PTEN as well as PI3K mutations. Furthermore, recent investigations have demonstrated ARID1A mutations in 40% of clear cell carcinomas of the endometrium. In addition, loss of expression of ARID1A has been identified in 26% of this group of tumors [28,32]. Studies by Le Gallow *et al* [64] have demonstrated that clear cell carcinomas may have molecular features of EEC or serous carcinomas as MSI and Tp53 mutations were detected. In addition, they identified mutations in TAF-1 or TATA-box binding associated factor-1. Additional studies need to be undertaken to fully understand the function of TAF-1 mutations in clear cell carcinomas [63].

4.3. Carcinosarcomas and undifferentiated carcinomas

Most carcinosarcomas have a molecular profile of serous carcinomas characterised by mutations in p53 and PPP2R1A. A small percentage of carcinosarcomas show mutations in PTEN, ARID1A, KRAS, POLE and microsatellite instability suggesting origin from endometrioid carcinomas [58]. Undifferentiated carcinomas are a molecularly diverse group of tumors and have demonstrated mutations from the four molecular subgroups described by TCGA [58]. (See Table 1.)

5. Lynch Syndrome

Lynch Syndrome (LS), or Hereditary Non-Polyposis Colorectal Carcinoma, (HNPCC) is an autosomal dominant syndrome that is one of the most commonly inherited tumor syndromes in humans. It results from mutations in the DNA MMR system which predisposes affected individuals to endometrial, ovarian, colorectal, urothelial, gastric and pancreaticobiliary carcinomas as well skin and brain tumors [36-41,65]. In order for LS to clinically manifest, both alleles must be inactivated; in accordance with Knudson's two-hit hypothesis [41]. The first hit in LS carriers is a mutated, germline allele in any of the mismatch repair genes, together with a wild-type allele [66]. The wild-type allele in LS carriers may then suffer a second hit by way of hypermethylation, mutation, or loss of heterozygosity which culminates in a dysfunctional DNA mismatch repair system. Microsatellite instability thus continues unchecked, with an increase in mismatches providing a fertile soil in which carcinomas may develop [66]. In sporadically occurring tumors, alleles of somatic neoplastic progenitor cell alleles are altered by hits which result in tumorigenesis. Either one or both hits may be inherited or can arise from epigenetic phenomena. In general, both hits are genetic in LS associated neoplasms. The first hit may be due to point mutations or due to larger nucleotide changes. Gene changes or loss of the wild-type or second allele are responsible for the second hit [41]. An epimutation, which is an anomalous inhibition of an active gene, [67] may occur as the first hit followed by promoter hypermethylation as the second hit. Promoter hypermethylation is a far more common form of inactivation in contrast to mutations and thus illustrates the importance of epigenetic phenomena [41].

Approximately half the number of females with LS may have endometrial carcinoma as their initial tumor and are susceptible to metachronous and synchronous neoplasms [38]. Thus, detection of LS patients is imperative so as to observe and investigate for different possible tumors in the affected individual. In addition, it affords the opportunity for genetic counselling and possible genetic testing and preventative measures for immediate relatives of the index patient [38,41,66]. Whilst the Amsterdam Criteria I and II, together with Bethesda Guidelines [37,43,68] may indicate the likelihood of a person having LS, these may be inadequate tools as they rely heavily on patients' knowledge of their own tumors as well as malignancies in relatives, in addition to having knowledge of the age of onset of the neoplasias.

5.1. LS history and syndromic terminology

The first family identified with LS dates to 1913, where after family history together with clinical findings were the basis for suspecting LS, until the first locus predisposing to this syndrome was recognized eighty years later [37,41]. Subsequently, an abundance of mutations associated with LS have been identified. Of late, epigenetic mechanisms such as DNA hypermethylation and chromatin remodelling have been shown to play a role in the pathogenesis of tumors [41].

HNPCC was the term initially coined to distinguish a newly identified hereditary cancer syndrome from the established Familial Adenomatous Polyposis (FAP) syndrome which is characterised by an abundance of colonic polyps and an early age of onset [69].

In 1991, the first set of criteria for HNPCC; the Amsterdam criteria; were published following combined discussions between clinicians and researchers. This set of criteria identified families who did not have FAP, but in whom three closely related people in a minimum of two generations had colonic carcinoma, with at least one of these diagnoses having been made under the age of 50 [69]. With the passage of time, more LS associated tumors were identified occurring outside of the colorectum. These extracolonic neoplasms were included in the revised

Table 1
Summarizes the molecular features identified in the main histological subtypes of endometrial carcinomas.

Histological subtype	Main molecular features
Endometrioid endometrial carcinomas	PTEN inactivation, PIK3CA mutation, microsatellite instability, KRAS mutation, RASSFIA promoter hypermethylation, CTNNB1 mutations, SPRY2 inactivation and FGFR2 Mutations (mutually exclusive with KRAS) and ARID1 mutations
Undifferentiated and De-differentiated carcinomas	Molecular diverse group of tumors. Upregulation of epithelial-mesenchymal transition associated proteins ZEB-1, SNAIL and TWIST. Downregulation of E-Cadherin.
Serous carcinomas	p53 mutation, Her2/neu over-expression, CDKN2A/p16 mutations or promoter hypermethylation, loss of heterozygosity of CDH1 encoding for E-Cadherin, PPP2R1A mutation, CCNE amplification, chromosomal instability, PIK3CA amplification
Clear cell carcinomas	PTEN, PI3K, ARID1, p53, TATA-box binding associated factor-1 mutations and microsatellite instability.
Carcinosarcomas	p53 and PPP2R1A mutations, upregulation of epithelial-mesenchymal transition associated proteins ZEB-1, SNAIL and TWIST.

1999 Amsterdam II criteria [69]. The Amsterdam criteria were shown to be limiting and did not identify a significant number of individuals afflicted by MMR mutations [39]. The Bethesda Guidelines were subsequently introduced in an attempt to rectify the shortcomings of the Amsterdam I and II criteria. [39,43,68,70-72]

Whilst the Amsterdam II criteria included non-colonic tumors such as endometrial carcinoma, they did not consider patients who had their first tumor being located in the female genital tract and did not consider the possibility of small family lineages. [73] As such, the revised Amsterdam criteria were not entirely useful to those afflicted by tumors of the female genital tract [73]. Both the Amsterdam and Bethesda criteria were mainly devised to target patients with colorectal tumors [73]. It is therefore difficult to assess the efficacy of these criteria with regard to tumors of the female genital tract. Clinically, the present Bethesda guideline is the best modality to assess the possibility of a patient having a tumor that may require subsequent genetic work-up for a suspected inherited tumor syndrome [73]. A shortcoming of this set of criteria is the lowered sensitivity in patient families that are small and those who have endometrial tumors as their main tumor type [73].

Lynch Syndrome is named after the physician Dr. Henry T. Lynch who was a doyen in identifying patients with this tumor syndrome. Since 1984, Lynch syndrome is the term used preferentially to HNPCC as the latter does not take into consideration the presence of extracolonic tumors [68-70]. Additionally, it should be borne in mind that HNPCC is in fact a clinical diagnosis that is made in patients who meet either Amsterdam I or II criteria. In contrast however, a diagnosis of LS is made following molecular evidence of a mutation in one of the MMR genes [71].

5.2. Molecular LS screening tests

5.2.1. Mismatch repair identification by immunohistochemistry

Immunohistochemistry targeting the four MMR proteins is a cost-effective method of detecting possible MMR defects [71]. Absolute negativity of tumor nuclei in the presence of suitable internal controls such as endothelial cells, lymphocytes and stromal cells is accepted as a valid result [43]. Therefore, faint, focal positive nuclear positivity is construed as demonstrating retention of staining (Fig. 3). As IHC for the four markers is a screening test, it is not diagnostic of LS, however, IHC has demonstrated a 94% sensitivity of detecting germline mutations [74].

5.2.2. Polymerase chain reaction (PCR) microsatellite instability

Microsatellite instability by PCR requires that neoplastic and non-neoplastic tissue from an individual be tested [66]. A number of markers are currently employed for MSI testing. The deletion or insertion of repeated nucleotides with resultant shortening or lengthening of DNA sequences typify microsatellite instability [75]. Following PCR, the fragment sizes are evaluated using gel electrophoresis. PCR products of different sizes from tumor are compared to the patient's normal tissue [42]. Tumors that have an allelic shift in two or more markers out of a panel of five are interpreted as having a high frequency of microsatellite instability or MSI-H [43,65,75,76]. Tumors that show an allelic shift in one of the markers (20%) are construed as having a low frequency of microsatellite instability (MSI-L); whilst tumors that do not show any allelic shift are interpreted as microsatellite stable (MSS) [43,66,75,76].

MSI PCR assesses function of MMR genes and may be able to document MSI cases that result from missense mutations in MMR genes. Such mutations may be missed immunohistochemically as they may demonstrate nuclear positivity [77].

5.2.3. Diagnosis of Lynch Syndrome

Whilst MMR IHC and MSI by PCR, hypermethylation studies and BRAF mutational assessment may suggest that an individual has LS, these are screening tools and are not diagnostic. LS is confirmed by the identification of germline mutations in one of the mismatch repair genes by sequencing [66,70].

5.2.4. Clinical correlates of various mutations

Phenotypically, LS is dominant but with variable expression [69]. The amount of MMR gene and protein impact on the clinical phenotype such that heterozygosity for an MMR gene may give rise to Lynch Syndrome but homozygosity results in Constitutional Mismatch Repair Deficiency Syndrome or CMMR-D, which is a rare recessive condition occurring in people who receive abnormal alleles from both parents [41]. The particular gene affected and the sex of an individual have an effect on the type of LS associated tumor that may arise [69]. Females with LS have a risk of up to 60% of developing endometrial carcinoma as their sentinel tumor [42]. In addition, females with LS have a greater lifetime likelihood of developing endometrial carcinoma than colorectal carcinoma [42]. The two genes that have lower penetration, MSH6 and PMS2, have of late been found to be responsible for an increased number of LS than in the past [69].

It has been estimated that between 70 and 90% of LS cases may be ascribed to mutations in either MSH2 or MLH1 [69]. The residual 10–30% of LS cases are due to PMS2 and MSH6 mutations [69]. A miniscule fraction of LS (up to 3%) may be attributed to mutations of the EPCAM gene which has a role in cell proliferation and signalling as well as cell adhesion. This gene is located immediately upstream of MSH2 and it has been shown that hypermethylation of the MSH2 promoter occurs following on deletions occurring in EPCAM's 3′ end [69].

It is now accepted that in LS, mutations of certain genes have a greater possibility of resultant carcinoma types [69]. Mutations in the major MMR genes, MLH1 and MSH2, are associated with an early age of onset (below 45 years of age), a high degree of microsatellite instability and high penetrance; all of which point toward an aggressive form of LS [39]. This may be a consequence of MLH1 and MSH2 being the stabilising component of their binding complex. Accordingly, a loss in either of these results in destabilisation of their binding partners.

Mutations in either PMS2 or MLH1 have the greatest likelihood and widest assortment of tumors ascribed to LS [69]. It has been shown that mutations in MLH1 and MSH2 have a fairly comparable probability of developing colorectal carcinoma [39]. However, individuals who have mutant forms of MSH2 have a greater possibility of developing endometrial, ovarian, renal and gastric carcinomas than individuals who have MLH1 mutations [39]. Of note, males carrying a mutant MSH2 gene have been identified as having the greatest risk for various tumors [69]. MMR mutation frequencies for endometrial carcinomas are between 24 and 40% for MLH1 and between 50 and 66% for MSH2 [42]. MSH2 mutations are the most commonly occurring MMR mutation identified in Lynch Syndrome associated endometrioid endometrial carcinoma [66,78].

Mutations in the minor genes result in a varied clinical picture. Aberrations in the PMS2 gene result in early neoplasia. Mutations in PMS2 have been associated with multiple colorectal adenomas as well as glioblastomas (Turcot syndrome) [39]. The occurrence of glioblastomas is likely attributable to increased mutations in tumor suppressor genes and oncogenes which have a higher degree of expression in the brain [39]. Individuals with PMS2 mutations have a lower risk of developing Lynch Syndrome associated neoplasms in particular, colorectal and female genital tract malignancies [65,69]. MMR mutational frequency for PMS2 in endometrial carcinomas is below 5% [42].

It has been shown that individuals with MSH6 mutations are less likely to be afflicted by colorectal carcinoma but have a significant risk of developing tumors of the female genital tract; in particular endometrial carcinoma [36,66,69]. Conklin and Longacre [66] state that MSH6 mutations in endometrial carcinoma occur at a lower frequency than compared to MSH2, whilst studies by Buchanan *et al* [79] and Devlin *et al* [49] have shown that mutations of MSH6 are the most common of the four mutations in patients with endometrial carcinoma. MSH6 mutations are strongly associated with the development

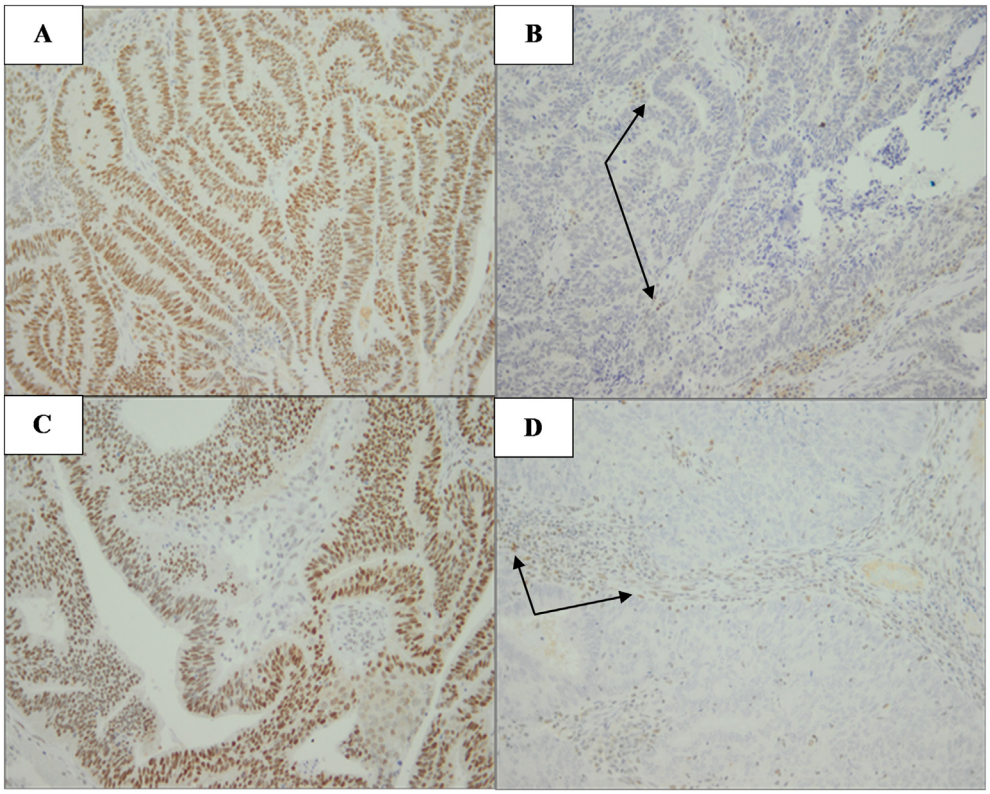

Fig. 3. Immunohistochemistry on endometrial carcinomas. 3A shows positive staining of tumor nuclei. 4 μm PMS2 stained section. 3B shows lymphocytes and stromal cell nuclei staining positively (arrows). The tumor nuclei are negative in this tumor. 4 μm PMS2 stained section. 3C shows a positive nuclear signal in the tumor cells. 4 μm MSH6 stained section. 3D shows lymphocytes and stromal cell nuclei staining positively (arrows). The tumor nuclei are negative in this tumor. 4 μm MSH6 stained section. Original magnification 2A-D: 200×.

endometrial carcinoma and there is a risk of between 64 and 71% of developing endometrial carcinoma with MSH6 mutations *versus* a relative risk of between 4 and 50% with MSH2 mutations [66]. Females harbouring mutations in either MSH6 or MSH2 are at greatest risk for endometrial carcinoma and have an overall estimated lifetime risk of 44% [69]. MSH6 mutations are believed to place affected individuals at specific risk for endometrial carcinoma and have a lifetime risk of up to 70% [42,68]. Studies have demonstrated that females with an MSH6 mutation present with endometrial carcinomas at an older age, typically over the age of 50 years [42,43,50,71,80-82].

5.2.5. *The evolving realm of LS tumors*

Whilst the Amsterdam I criteria only included colorectal carcinomas, the Amsterdam II criteria broadened the spectrum of neoplasms to include endometrial, renal pelvis, ureter and small bowel based on information that was available at the time which indicated that these tumors had the greatest likelihood of occurrence amongst all LS tumors [41]. The realm of LS associated tumors has evolved with time and in different locations whereby Korean families have a greater incidence of pancreatic and gastric carcinomas but lower incidence of endometrial carcinomas in contrast to families of Dutch ancestry [41]. This is further evidenced by the high occurrence of breast carcinomas in Brazilian families relative to patients of other nationalities [41]. These findings suggest that the environment has a bearing on the types of tumors that develop in LS.

It has been suggested that LS tumors should fulfil the following two criteria:

1. The lifetime relative risk of the neoplasms should be greater in individuals with the mutation than the rest of the population
2. The tumors should occur due to the genetic aberration that the patient has [41,69].

5.2.6. *Lynch-like syndrome*

There are cases in which negative staining may be identified on MMR immunohistochemistry or there may be evidence of microsatellite instability by PCR but subsequent germline testing proves negative [70]. Patients in whom this has been identified are known to have “Lynch-like Syndrome” [70,83]. Unlike sporadically occurring microsatellite carcinomas, distinction of LS patients from Lynch-like Syndrome is an incredulous task as Lynch-like Syndrome patients have microsatellite instability of their tumors [84]. In addition, the tumors are negative for MMR by immunohistochemistry for MLH1 as well as for the other MMR stains as may be seen in true Lynch Syndrome. Furthermore, Lynch-like Syndrome patients have a similar age at which tumors manifest. Unlike LS, however, patients with Lynch-like syndrome do not have an identifiable germline mutation [84,85] Three potential explanations for this have been considered. The first is that there may be unidentified gene mutations that result in microsatellite instability besides MMR genes. [84,86] Secondly, there may be germline mutations in MMR genes that are not detected by current modalities and thirdly there may be bi-allelic MLH1 hypermethylation or other genomic aberrations besides MMR mutations that culminate in microsatellite unstable neoplasms [84]. It is plausible that all three mechanisms may have a role to play in Lynch-like Syndrome as they are not mutually exclusive processes [84]. The first potential explanation may not be a probable one, as the mismatch repair system has been extensively researched and besides EPCAM mutations, to date, there are no other identifiable germline mutations. The second possible explanation is a conceivable one. The current detection methods sequence exonic regions of these genes and identify some commonly occurring deletions for MLH1 and MSH2. Mutations in EPCAM were identified as orchestrating methylation of the adjacent residing MSH2. It is thus a definite possibility that different mechanisms may be discovered that have an impact on MMR genes. Currently, intronic and promoter regions do not routinely undergo mutational analysis and understanding by the scientific community is not finalised or perfect and as such, the possibility of mutations yet to be discovered within MMR DNA is a very viable one. This could result in a re-categorization of patients from Lynch-like to Lynch Syndrome [84]. Sourrouille *et al* [87] demonstrated

the occurrence of somatic mutations on both alleles resulting in inactivation of MMR genes. The third possibility has been the subject of studies undertaken by Mesenkamp et al. [83] This group of researchers suggest that in approximately 50% of MMR negative tumors that were shown to be negative for both MLH1 promoter hypermethylation and germline mutations of MMR genes, somatic mutations could be identified in either MSH2 or MLH1 [83]. Boland [88] suggests that somatic mutations are also likely to occur in MSH6 and PMS2 [88]. Studies by Mesenkamp *et al* [83] illustrate that two somatic mutations have an impact on MMR gene expression [83]. Furthermore, their work suggests that tumors in patients with Lynch-like syndrome arise at an earlier age than sporadically arising microsatellite unstable tumors. Research undertaken by this group of investigators showed that the average age of tumor diagnosis in patients who had two somatic mutations was virtually the same as those who were affected by germline mutations in MHS2 or MLH1 but younger than in patients who developed sporadic tumors [83,84,89,90]. The implication of this in patients who have two somatic mutations, in addition to the group of Lynch-like Syndrome patients who do not have two identifiable mutations, is that these individuals may well have Lynch Syndrome [83,84]. It is thus incumbent on the treating physician to continue surveillance for tumor development in these patients. Identification of sporadically occurring tumors from those occurring in Lynch Syndrome with its high prevalence of neoplasia is important; but currently the distinction between LS and Lynch-like Syndrome is not as clear. Inactivation of MMR genes are a result of various processes such as germline mutation, somatic mutation of MMR genes and hypermethylation as a form of epigenetic phenomena. It is possible that these mechanisms may be taking place in Lynch-like Syndrome. Furthermore, the possibility exists that Lynch-like Syndrome patients have aberrations that are a mixture of those seen in sporadic tumor and Lynch Syndrome. Additional research into this group of patients will undoubtedly yield greater insight into the best treatment plans [84].

Mesenkamp *et al* [83] state that tumors in patients with somatic mutations in the absence of hypermethylation and germline mutations need not be referred to as having Lynch-like Syndrome and that these individuals and their relatives may follow surveillance programmes according to their family history. This then removes the required surveillance for extra-colonic tumors and also reduces the need for earlier onset of surveillance colonoscopies [83].

6. Screening programs

There are several institutions in the United States of America that have implemented LS screening in patients with endometrial carcinomas. This however, is not a universally accepted practice [91]. It has been suggested that females with endometrial carcinoma under the age of 60 years have their tumors tested for mismatch repair by the four MMR antibodies. If these stains all show retained positive nuclear staining, then no further testing is required; or if clinical concern for LS persists, then the patients may be referred for genetic counselling and testing. If there is negative staining for MLH1 alone or MLH1 and PMS2, then testing for MLH1 hypermethylation should be performed. If methylation is positive, this points to a sporadic tumor; whereas negative methylation suggests the possibility of LS and thus the patient should be referred for germline testing and genetic counselling. Negative staining on PMS2, MSH2 or MSH6 alone or MSH6 and MSH2 suggest possible LS and thus the patient should be seen by a genetic counsellor and germline mutational analysis performed. Selective screening of patients over the age of 60 years may be undertaken in cases where there is high clinical suspicion for LS [91]. Such screening programmes may thus detect patients with LS and allow for prevention of the development of other LS-associated neoplasms. [91]

7. Conclusion

Endometrial carcinomas are frequently seen by practising histopathologists. There are numerous instances whereby a morphological distinction between rigid categorization of Type I and II tumors is difficult and not practical. Utilising immunohistochemical stains and where available, sequencing for POLE in endometrial tumors, in centres where this is available, as outlined by studies undertaken by Stelloo *et al* [20] and Talhouk *et al* [15] may facilitate the identification of the four molecular subtypes as demonstrated by the TCGA [17].

The molecular classification of endometrial tumors is undoubtedly exciting as this now allows for further targeted therapeutic options for patients with endometrial carcinomas and may reduce overtreatment in patients who have a more favourable molecular profile. Furthermore, ancillary studies such as immunohistochemistry and PCR may identify patients who may harbour germline mutations of Lynch Syndrome. Recent studies suggest that patients with mismatch repair deficiencies may benefit from an antibody to Programmed cell death-1 (PD-1) which is expressed on activated T-cells. The binding of the antibody to PD-1 blocks immune tolerance which allows for heightened host T-cell activity against tumor cells. [92] Thus, undertaking IHC and PCR testing may bring to the fore patients who may not meet Amsterdam or Bethesda criteria, and may allow for these patients to undergo formal germline mutational testing. This may then prevent additional tumors in the affected individual as well as facilitate screening in her kindred. In addition, patients with microsatellite unstable tumors may be candidates for emerging adjuvant treatment modalities [92].

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