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Molecular genetic heterogeneity in undifferentiated endometrial carcinomas

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Undifferentiated and dedifferentiated endometrial carcinomas are rare and highly aggressive subtypes of uterine cancer, not well characterized at a molecular level. To investigate whether dedifferentiated carcinomas carry molecular genetic alterations similar to those of pure undifferentiated carcinomas, and to gain insight into the pathogenesis of these tumors, we selected a cohort of 18 undifferentiated endometrial carcinomas, 8 of them with a well-differentiated endometrioid carcinoma component (dedifferentiated endometrioid carcinomas), and studied them by immunohistochemistry and massive parallel and Sanger sequencing. Whole-exome sequencing of the endometrioid and undifferentiated components, as well as normal myometrium, was also carried out in one case. According to The Cancer Genome Atlas classification, we distributed 95% of the undifferentiated carcinomas in this series as follows: (a) hypermutated tumors with loss of any mismatch repair protein expression and microsatellite instability (eight cases, 45%); (b) ultramutated carcinomas carrying mutations in the exonuclease domain of *POLE* (two cases, 11%); (c) high copy number alterations (copy-number high) tumors group exhibiting only *TP53* mutations and high number of alterations detected by FISH (two cases, 11%); and (d) low copy number alterations (copy-number low) tumors with molecular alterations typical of endometrioid endometrial carcinomas (five cases, 28%). Two of the latter cases, however, also had *TP53* mutations and higher number of alterations detected by FISH and could have progressed to a copy-number high phenotype. Most dedifferentiated carcinomas belonged to the hypermutated group, whereas pure undifferentiated carcinomas shared molecular genetic alterations with copy-number low or copy-number high tumors. These results indicate that undifferentiated and dedifferentiated endometrial carcinomas are molecularly heterogeneous tumors, which may have prognostic value.
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Undifferentiated endometrial carcinoma is a rare but highly aggressive subtype of endometrial carcinoma,[1] representing approximately 9% of endometrial carcinomas.[2] It is often misdiagnosed as grade 3 endometrioid carcinoma. Defining features include: (a) monotonous medium or large-sized cells; (b) diffuse pattern of growth; and (c) a lack of appreciable glandular, papillary, squamous, or neuroendocrine differentiation.[1,3,4] Undifferentiated endometrial carcinoma lacks expression of epithelial markers, such as keratins, E-cadherin, and mir-200 but express ZEB-1, a well-known repressor of E-cadherin.[5] In addition, undifferentiated endometrial carcinoma is frequently associated with mismatch repair deficiency.[5]

Occasionally, undifferentiated endometrial carcinoma is associated with a low-grade endometrioid

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carcinoma component, hence it is referred to as 'dedifferentiated endometrioid endometrial carcinoma'.[2] In a recent study,[6] a common origin of both components was proposed as all driver mutations present in the endometrioid component were also found in the undifferentiated (dedifferentiated) component. In that study, most mutations were found in genes such as *PTEN* (40%), *PIK3CA* (50%), *CTNNB1* (30%), and *TP53* (30%), which are frequently involved in the development of endometrioid carcinomas.[6]

However, it is still unclear whether pure undifferentiated carcinomas differ molecularly from so-called dedifferentiated carcinomas.

Recently, The Cancer Genome Atlas (TCGA) Research Network reported an integrated molecular analysis of 373 endometrial carcinomas, which were evaluated at the genomic, exomic, transcriptomic, epigenomic, and protein levels. This analysis showed that endometrioid and serous carcinomas can be classified into four distinct molecular categories: hypermutated microsatellite unstable, *POLE* ultramutated, copy-number low/microsatellite stable and copy-number high/'serous-like'. The hypermutated, ultramutated and copy-number low subgroups were represented predominantly by endometrioid tumors whereas the copy-number high/'serous-like' subgroup was composed of both serous and high-grade endometrioid subtypes. Mixed endometrial carcinomas were assigned to the copy-number low (endometrioid) and the copy-number high ('serous-like') tumors, indicating the molecular genetic diversity of these neoplasms. However, no mixed undifferentiated/well-differentiated (dedifferentiated) carcinomas were included in the TCGA study. Finally, a less sophisticated and practical way of assigning endometrial carcinomas to the TCGA categories, based on immunohistochemical analysis of mismatch repair proteins and *TP53* and *POLE* mutational analyses, has been proposed.[7]

In the current study, we investigated whether undifferentiated and dedifferentiated carcinomas share molecular genetic alterations and they represent a molecularly homogenous group of tumors. To this end, we selected a cohort of 18 undifferentiated endometrial carcinomas, 8 of them with an endometrioid carcinoma component, to be massively sequenced using the Haloplex Cancer Research Panel Enrichment Kit and Ion Torrent sequencing platform. In addition, one case was subjected to whole-exome sequencing for comparing the genetic alterations of the endometrioid and the undifferentiated elements.

Materials and methods

Case Selection

This study includes a total of 22 endometrial carcinomas, diagnosed initially as undifferentiated carcinomas, from the files of the Departments of Pathology of Hospital Universitario Ramón y Cajal, Madrid; Massachusetts General Hospital, Boston; Memorial Sloan Kettering Cancer Center, New York; Hospital de la Santa Creu i Sant Pau, Barcelona; and Hospital Universitari Arnau de Vilanova, Lleida). Clinicopathological features are presented in Supplementary Table S1. The pathological and immunohistochemical features of 10 cases have been previously reported in part.[5] The diagnosis of undifferentiated carcinoma was based not only on conventional morphological features but also on the characteristic immunohistochemical pattern reported previously; ie, the absence of E-cadherin expression together with ZEB1 nuclear immunoreaction (see Figures 1 and 2).[8] Two of the 22 tumors were reclassified as grade 3 endometrioid carcinomas containing areas of well-differentiated carcinoma. In these two cases, the solid component, interpreted initially as undifferentiated carcinoma, expressed membranous E-cadherin and lacked the nuclear ZEB1 immunoreaction. Two other cases were reclassified as carcinosarcomas with areas of undifferentiated carcinoma. The study was approved by the Institutional Review Board of the Hospital Ramón y Cajal and by the other participating medical institutions.

Immunohistochemistry

The following antibodies were used: E-cadherin (cat. no. IR059; DAKO, Glostrup, Denmark; ready to use) and ZEB1 (cat. no. ab87280; ABCAM, Cambridge, UK; dilution 1:300), p53: (cat. no. IR616; DAKO, ready to use), MLH1 (cat. no. M3640; DAKO, ready to use), PMS2 (cat. no. IR087; DAKO, ready to use), MSH2 (cat. no. M3639; DAKO, ready to use), MSH6 (cat. no. M3646; DAKO, ready to use), β-catenin (cat. no. 0001109QD; Master Diagnóstica, Granada, Spain; ready to use), ARID1A (cat. no. HPA005456; Sigma, St Louis, USA; dilution 1:500), and SMARCB1 (BD Biosciences, Franklin Lakes, NJ USA; dilution: 1/100). Immunostaining was performed using the EnVision detection system (K5007, Dako).

Massive Parallel Sequencing

Haloplex Cancer Research Panel was used as the enrichment kit. This panel targeted 1205 hotspots in 199 regions from 47 genes, including most frequently mutated genes in endometrioid endometrial carcinoma (*PTEN*, *PIK3CA*, *PIK3R1*, *KRAS*, *CTNNB1*, *FGFR2*, or *TP53*), with the exception of *ARID1A*. In order to know whether targeted regions overlapped with mutations found in TCGA study,[9] we calculated the percentage of variants located across covered regions and across hotspot regions ± 30 bp. Sequencing of libraries was performed on Ion Torrent sequencing platform in four chips class 316 following standard procedures, expecting an average of

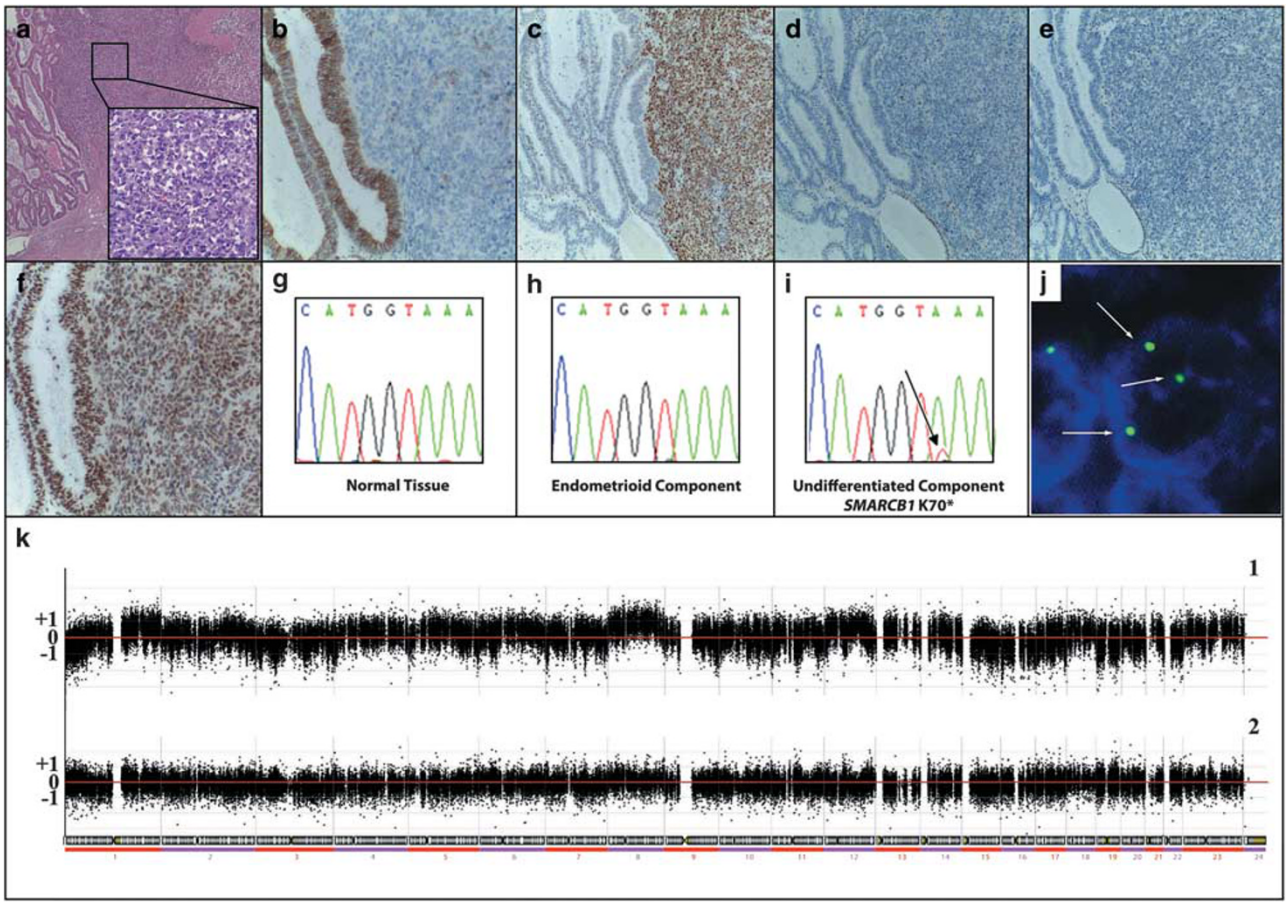

Figure 1 Molecular characteristics of UEC-14, a dedifferentiated endometrioid carcinoma with mismatch repair and ARID1A deficiency. (**a**) Hematoxylin–eosin staining showing a well-differentiated component besides an undifferentiated component. Magnified image showed the rhabdoid features of the undifferentiated component. (**b**) E-cadherin staining positive for differentiated component, negative for undifferentiated component. (**c**) ZEB-1 staining positive for undifferentiated component and negative for differentiated component. (**d**) Negative MLH-1 staining. (**e**) Negative ARID1A staining. (**f**) Positive SMARCB1 staining. (**g**) Wild-type *SMARCB1* (chr22:24134051-24134059) sequence obtained for UEC-14 normal component. (**h**) Wild-type *SMARCB1* sequence obtained for UEC-14 endometrioid component. (**i**) *SMARCB1* variant (chr22:24134057A->T) found in UEC-14 undifferentiated component identified by exome sequencing. Observed low variant allele frequency agreed with NGS results (~18%). (**j**) Confirmation of chr8 trisomy constrained to undifferentiated component by FISH using a chr8-centromere probe. (**k**) Lod-score value of the depth obtained from Whole Exome Sequencing. 0 value determines the diploid status of the chromosome in the tumoural components. Gain of chr8 is clearly visible and was confirmed by FISH.

25 Mb per sample (around 17 Mb per sample were recommended by Haloplex Protocol).

To analyze sequencing results and to avoid an unacceptable false negative rate, a bioinformatics analysis pipeline was developed using TMAP (https://github.com/iontorrent/TS/tree/master/Analysis/TMAP) as aligner and VarScan[10] as variant-caller, with no filters. Variant annotation was performed using the VEP from Ensembl with version 74 of the human reference genome (http://www.ensembl.org/info/docs/tools/vep/index.html). Variants were then filtered using the functional information (selecting only deleterious variants), the variant allele frequency (>0.15), and the strand-bias from both the variant and the reference allele. In the case of having normal tissue available, those variants present in the normal component were ruled out. Taking into account the information from Sanger sequencing, visual inspection of variants was performed using IGV browser[11] as the final selection step.

FISH Analyses

In 11 tumors, interphase fluorescence *in situ* hybridization (FISH) analysis was performed using commercial probes delineating several loci on different chromosomal regions (see Supplementary Table S2). In dedifferentiated tumors, FISH analysis was limited to the undifferentiated component. Pretreatment of slides, hybridization, posthybridization processing, and signal detection were performed as reported elsewhere.[12] Only samples showing sufficient FISH efficiency ($>90\%$ nuclei with signals) were evaluated. Signals were scored in at least 50 not overlapping, intact nuclei. Non-neoplastic cells present in the section were used as a control. Results were interpreted as follows: (a) gain: when the ratio between gene and control probe signals was between 1 and 2.5 (both excluded); (b) amplification: when the ratio between gene and control probe signals was ≥ 2.5; (c) deletion: when the ratio between gene and

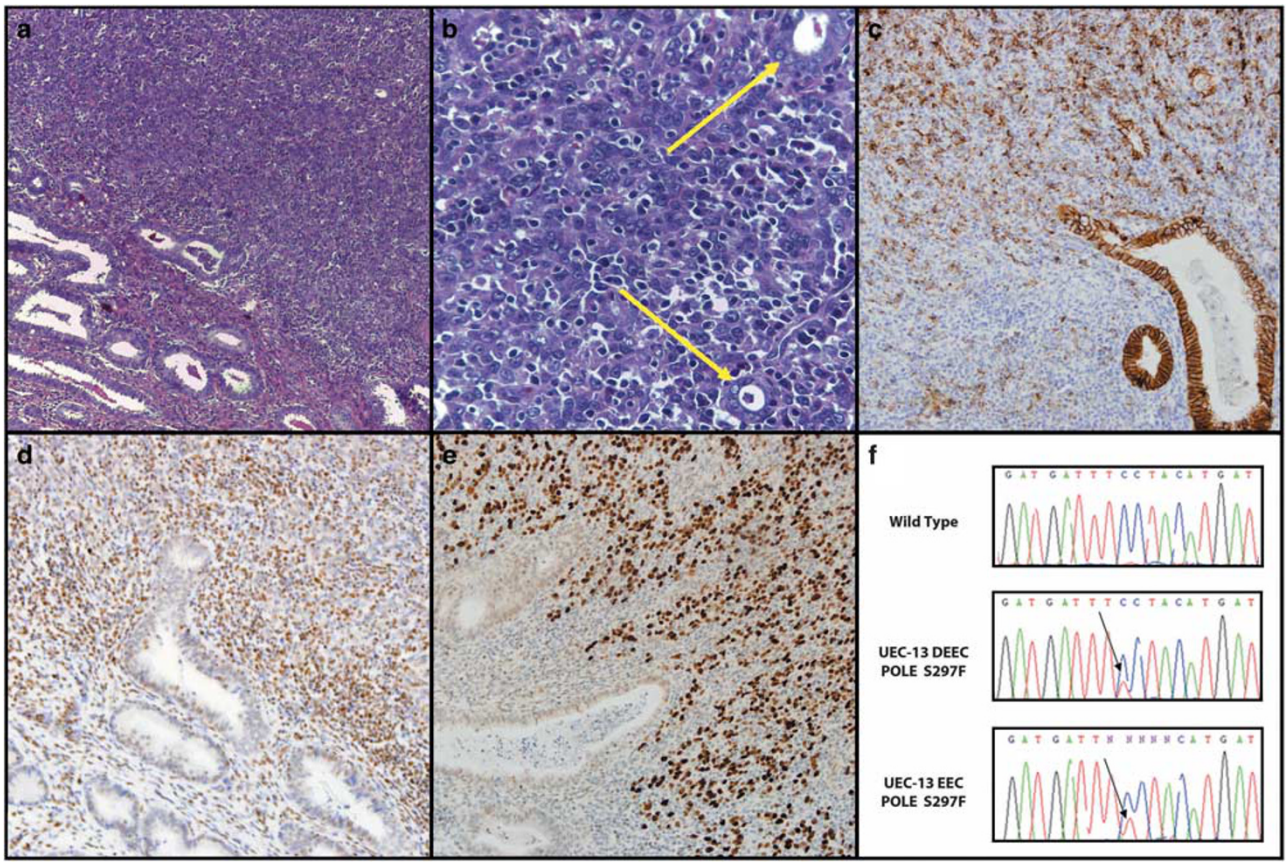

Figure 2 Molecular characteristics of UEC-13, a dedifferentiated endometrioid carcinoma with *POLE* and *TP53* mutations. (**a**) Hematoxylin–eosin staining showing a well-differentiated component beside undifferentiated component. (**b**) Hematoxylin–eosin staining showing a field within the undifferentiated component with two glands (yellow arrows). (**c**) Progressive loss of E-cadherin expression throughout undifferentiated component. (**d**) Focal gain of ZEB-1 expression throughout undifferentiated component. (**e**) TP53 staining positive for undifferentiated component and negative for differentiated component. (**f**) Chr12:133253143-133253160 sequence. From up to bottom: wild-type sequence, *POLE* S297F mutation identified in UEC-13 undifferentiated component and *POLE* S297F mutation identified in UEC-13 endometrioid component.

control probe signals was <1; (d) aneuploidy: the presence of only one gene and centromeric probes in $>50\%$ of cells evaluated was considered monosomy while the presence of ≥ 3 gene and centromeric probes was considered polisomy. For *EGFR* gene evaluation, the presence of three and four centromeric probes was considered trisomy and tetrasomy, respectively, and the presence of ≥ 5 centromeric and gene probes was considered polisomy.

Whole-Exome Analysis

To find out whether or not the mutational profile of the undifferentiated component of the mixed carcinomas differed from that of the differentiated component within an individual tumor, we performed whole-exome sequencing of both components, together with normal myometrial tissue (UEC-14), following technical specifications from Sistemas Genómicos S.L., on an Illumina Hiseq 2000 platform. The enrichment method was Agilent SureSelect Exome V4, targeting a total of 51 Mb. Bioinformatics analysis was performed as described above, with the exception of using Novoalign (http://www.novocraft.com/products/novoalign/) instead of TMAP in the alignment step. Somatic mutations were considered those present in the tumor but not in normal tissue. CONTRA package[13] was used to analyze the genome instability using the normal component data as control.

Sanger Sequencing

To validate massive parallel sequencing data, primers were designed to target different mutations in *PIK3CA*, *PIK3R1*, *ATM*, and *SMARCB1* (Supplementary Table S3). As Haloplex Cancer Research Panel lacks coverage of some *PTEN* mutations, we also designed 12 primer pairs (Supplementary Table S3) to amplify the 9 *PTEN*'s exons by PCR and perform Sanger sequencing for all cases. To further classify tumors within the TCGA ultramutated group,[9] we designed two pairs of primers (Supplementary Table S3) to sequence the two

usually mutated exons (exons 9 and 13) containing the protein catalytic domain of *POLE* in all cases.

Results

Pathological and Immunohistochemical Features

Of the 18 undifferentiated endometrial carcinomas, 7 were pure and the remaining 11 cases exhibited an endometrioid component (a grade 1–2 endometrioid carcinoma in 10 and a grade 3 endometrioid carcinoma in 1).

The most relevant clinical, pathological, inmunohistochemical, and molecular genetic features of all 22 tumors are presented in Supplementary Table S4. Here we describe the immunohistochemical and molecular features of the 18 undifferentiated/dedifferentiated endometrial carcinomas.

Every immunohistochemical essay was performed in the 7 pure undifferentiated cases, in the undifferentiated component of the 11 dedifferentiated cases, and in the differentiated component of 7 dedifferentiated cases. In the differentiated component of four dedifferentiated cases, IHQ essays were not possible to perform owing to the lack of dedifferentiated tissue in the preparations under study.

Diagnosis for undifferentiated status was confirmed as stated in Material and methods section: undifferentiated component showed lack of E-cadherin staining and nuclear staining of ZEB1, whereas differentiated component showed normal membrane staining of E-cadherin and absence of ZEB1 staining. Only in case UEC-5 this pattern was not confirmed owing to the lack of material.

Loss of ARID1A expression was observed in 10 out of the 18 cases (56%); being the loss total in 8 and focal in 2 cases, respectively. Noteworthy, the two cases with focal loss were dedifferentiated carcinomas and the loss of expression was limited almost exclusively to the undifferentiated component (in one case, a few areas of the differentiated endometrioid component had loss of ARID1A expression, see Supplementary Figure S1). Both cases also exhibited loss of MLH1/PMS2 expression across the entire tumor.

Loss of expression of at least one mismatch repair protein was found in eight tumors (45%): six showed loss of MLH1/PMS2, one loss of PMS2 only, and one loss of MSH2/MSH6.

p53 overexpression (at least in 75% of tumor cells) was found in 3 cases (17%). Noteworthy, in the dedifferentiated carcinoma, p53 overexpression was restricted only to the undifferentiated component.

Nuclear β-catenin immunoreaction was seen only in 1 case (6%) and was also confined to the undifferentiated component of a dedifferentiated carcinoma (Supplementary Figure S2).

All tumors were checked for SMARCB1 (INI1, BAF47) expression by immunohistochemistry. Normal nuclear SMARCB1 staining was found in all cases.

Mutation Analysis

We first compared the mutations covered by Haloplex Cancer Panel in *PTEN*, *PIK3CA*, *PIK3R1*, *KRAS*, *CTNNB1*, *FGFR2,* and *TP53* with those previously reported for endometrioid endometrial carcinomas, mainly by TCGA.[9] We found coverage $>90\%$ for all genes except for PTEN in which coverage reached 50% only (Supplementary Table S5). Accordingly, we performed Sanger sequencing for all nine PTEN exons.

For the 16 undifferentiated endometrial carcinomas subjected to massive parallel sequencing (Supplementary Table S6), the mean number of bases obtained per sample was 25 711 256, which allowed a median depth per sample of 40. However, the distribution of the depth was not homogeneous along the target regions and most of the depth was limited to the hotspot regions (median depth per sample of 236). We observed that this was due to the fact that larger amplicons could not be sequenced in most of the FFPE samples as several regions were not covered at all as designed amplicons of suitable-size were not available for Ion Torrent protocol (data not shown).

Taking into account the Haloplex and Sanger sequencing results, a total of 43 different pathogenic mutations were identified in the set of 16 undifferentiated carcinomas (Supplementary Table S4). Ten out of the 43 (23%) mutations were found in *PTEN*, and 4 of them were only detected by Sanger sequencing. Mutations were also detected in other genes commonly mutated in different types of endometrial carcinomas, such as *TP53* (4 out of 43, 9%), *PIK3R1* (3 out of 43, 7%), *PIK3CA* (3 out of 43, 7%), *KRAS* (2 out of 43, 5%), and *CTNNB1* (1 out of 43, 2%) (Supplementary Table S4). *POLE* mutational study showed that two tumors carried variants previously described as pathogenic and associated with ultramutated phenotype (Supplementary Table S4).[9,14–16]

Regarding phenotype–genotype correlations, only 1 pure undifferentiated carcinoma had mismatch repair deficiency in contrast to 7 out of the 11 dedifferentiated tumors. The other six pure undifferentiated carcinomas carried *PTEN* or *TP53* mutations as their main molecular alterations (Figure 3).

Chromosomal Alteration Detected by FISH

A total of 7 chromosomes and 11 genes were checked for alterations in 11 cases (6 pure undifferentiated and 5 dedifferentiated cases) (Supplementary Table S7). Pure undifferentiated cases with *TP53* mutations showed higher number of alterations (5.75 in average) compared with *TP53* wild-type cases (1 in average), which is consistent with previous results.[9] Noteworthy, one case with no identified molecular alteration showed the highest number of aberrations (three) among the *TP53* wild-type cases.

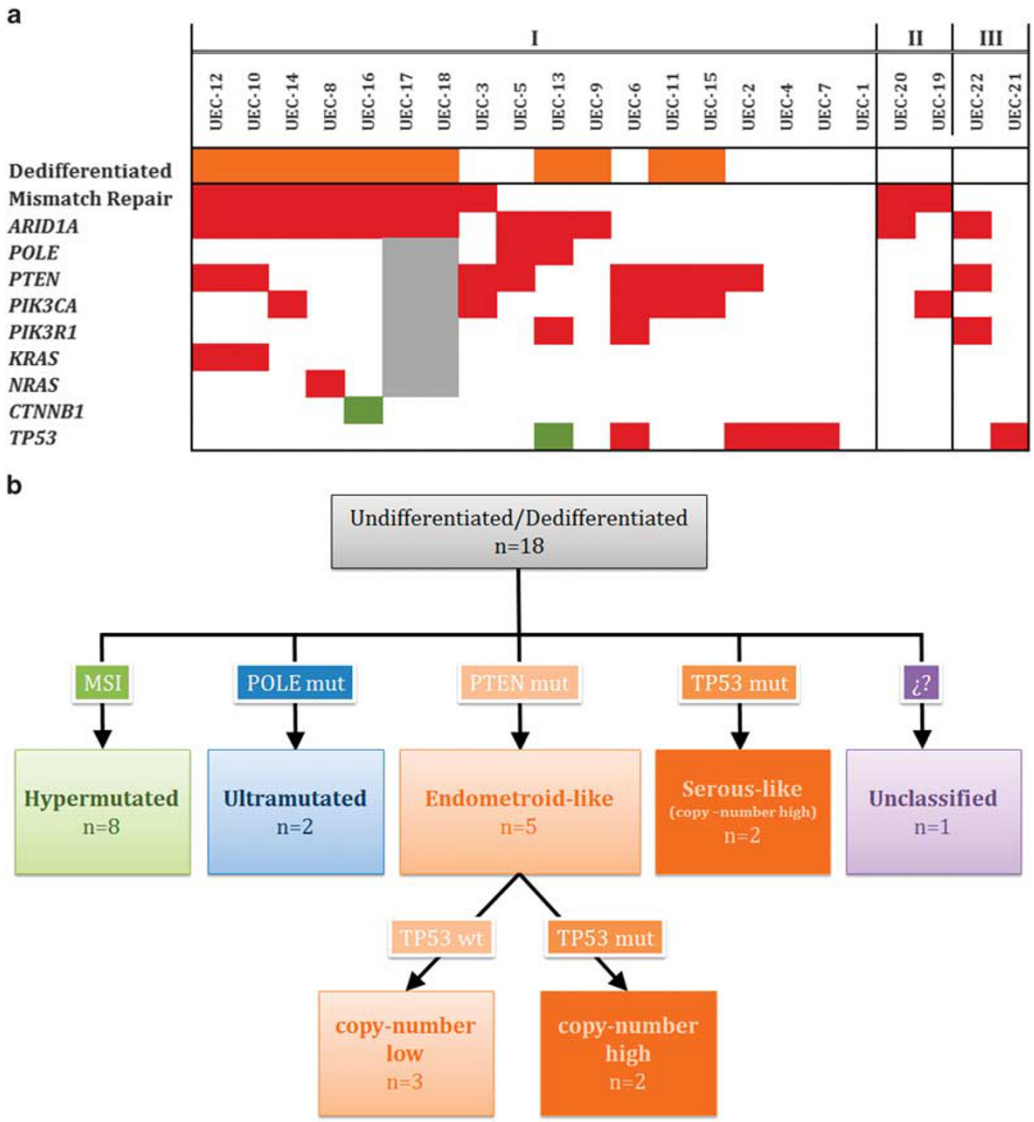

Figure 3 (**a**) Summary of the molecular characteristics observed in dedifferentiated, pure undifferentiated (I) and high-grade endometrioid carcinomas (II) and carcinosarcomas (III). Alteration for a gene (mutation or loss of expression) is indicated in red, no identified alteration for a gene (wild-type sequence or regular expression) in white and gene-not-analyzed for a sample in gray. (**b**) Schematic classification of the 18 undifferentiated/dedifferentiated tumors in this series according to TCGA molecular classification.

TCGA Based Molecular Classification of Undifferentiated/Dedifferentiated Carcinomas

According to the molecular classification scheme proposed by TCGA and following the simplified approach proposed in a recent study,[7] we classified 17 out of the18 undifferentiated carcinomas in this series as follows: 8 cases (45%) showing loss of any mismatch repair protein expression into the microsatellite instability hypermutated category; 2 cases (11%) carrying pathogenic mutations in the exonuclease domain of *POLE* into the ultramutated group; 2 cases (11%) showing only *TP53* mutations and high number of alterations detected by FISH into the copy-number high group; and 5 cases (28%) with molecular alterations typical of copy-number low endometrioid endometrial carcinomas. However, two of the latter cases showed additional mutations in *TP53* and higher number of alterations detected by FISH; therefore, these tumors could have progressed to a copy-number high phenotype (Figure 3b). In the remaining case, different chromosomal alterations were identified by FISH but no molecular alteration that allowed its classification was found.

Exome Sequencing

Whole-exome sequencing allowed the identification of somatic variants across most exonic regions in the genome of UEC-14. The final median depth across target regions was 38, 33, and 36, with 0.20, 0.17, and

0.25% of regions not covered at all (>90% of bases not covered for at least one read) for normal tissue, endometrioid, and undifferentiated carcinoma samples, respectively. A total of 123 somatic variants (40 indels and 83 substitutions) were identified for both tumor components (see genes affected in Supplementary Table S8). In addition, 44 somatic variants (19 indels and 25 substitutions) were identified only in the undifferentiated component, and 3 somatic variants (2 indels and 1 substitution) were exclusively found in the endometrioid component. Mutations identified by massive parallel sequencing were also identified by whole-exome sequencing. In agreement with the loss of expression of ARID1A in this case, two pathogenic mutations (Y1233* and 788delC) were detected in both endometrioid and undifferentiated components.

Genome instability study (see Figure 1k) showed a flat pattern for the differentiated component with no apparent alteration, whereas the pattern for the dedifferentiated component (see Figure 1k) showed trisomy of chromosome 8, partial loss of 1p36.33, and uncertain monosomy of chromosomes 15 and 16. Chromosome 8 trisomy was confirmed by FISH (see Figure 1j).

UEC-14 showed rhabdoid features in the undifferentiated component. As we found a heterozygous deleterious mutation in *SMARCB1* gene, we analyzed all 18 tumors for SMARCB1 (INI1, BAF47) expression by immunohistochemistry. As stated before, normal nuclear *SMARCB1* expression was found in all cases.

Discussion

The results of this study suggest that undifferentiated carcinomas may develop through any of the four molecular pathways described by TCGA for endometrial carcinomas; ie, hypermutated (mismatch repair deficiency), ultramutated (*POLE* mutated), copy-number low, and copy-number high (*TP53* mutated).[9] Nearly half (45%) of the undifferentiated carcinomas in this series belong to the hypermutated group, which contrasts with the frequency of microsatellite instability reported in most series of sporadic endometrial cancer (15–30%).[17] However, the frequency of mismatch repair deficiency seems to be higher in high-grade endometrioid carcinomas (45–63%),[5,18] suggesting an intrinsic potential for tumor progression of carcinomas from the hypermutated group.

A remarkable finding in this study was that two undifferentiated carcinomas carried *POLE* mutations that affect the activity of the catalytic subunit, involved in nuclear DNA replication and repair as described by TCGA.[9,14,15] Endometrioid carcinomas with *POLE* exonuclease domain mutations have been recently described to be frequently of high histological grade exhibiting morphological heterogeneity and ambiguity and tumor-infiltrating lymphocytes and/or peri-tumor lymphocytes.[19] One of the two ultramutated tumors in this series was a dedifferentiated carcinoma rich in tumor-infiltrating lymphocytes and the other was a pure undifferentiated carcinoma. The former tumor also had a *TP53* mutation confined to the undifferentiated component (Figure 2) similarly to the TCGA ultramutated carcinomas. A dedifferentiated carcinoma carrying *POLE* exonuclease domain mutations has recently been reported.[16] In this case, aberrant p53 immunoreaction was also confined to the undifferentiated component.

One third of undifferentiated carcinomas in our series derive from copy-number low endometrial carcinomas. In this group, the most frequent driver alteration was *PTEN* mutation, often associated with mutations in other genes of the same pathway (*PIK3CA*, *PIK3R1*) in endometrioid endometrial carcinomas.[9] However, 2 cases (11%) that lacked the characteristic molecular genetic alterations of endometrioid carcinomas had *TP53* mutations, suggesting that at least some undifferentiated carcinomas might develop through the 'serous-like' (copy-number high) pathway.

Regarding genotype–phenotype correlation and based on a relatively small number of cases, this study suggests that dedifferentiated carcinomas occur mainly in the setting of endometrioid carcinomas with microsatellite instability or *POLE* mutations, whereas most pure undifferentiated carcinomas develop from the copy-number low or 'serous-like' copy-number high tumors.

Another purpose of this study was to investigate the molecular mechanisms that allow 'dedifferentiation' of endometrioid carcinomas to undifferentiated carcinomas. The role of *CTNNB1*, *PPP2R1A*, and *TP53* in this phenomenon has been suggested by a previous report;[6] however, that report lacked information regarding mismatch repair proteins, ARID1A expression, and *POLE* mutation analysis. Our results suggest that different pathways may be followed depending on the molecular subtype (Figure 3). Thus tumors with mismatch repair deficiency (hypermutated) seem to acquire a dedifferentiated phenotype through accumulation of mutations in genes that are regularly altered in endometrioid carcinomas and not *TP53* mutations. Furthermore, our results suggest that *ARID1A* has a role in the progression of endometrial carcinomas with mismatch repair deficiency.[20] We found that 70% of tumors with loss of ARID1A had microsatellite instability and that loss of ARID1A occurred mainly in the undifferentiated component of two dedifferentiated carcinomas with mismatch repair deficiency in both components.[21] Similarly, in the case of one dedifferentiated carcinoma with microsatellite instability, we found *CTNNB1* mutations exclusively in the undifferentiated component (Supplementary Table S4).

On the other hand, tumors with *PTEN* mutations as main driver (copy-number low carcinomas) probably might acquire undifferentiated phenotype

through mutations in *TP53*, as such mutations were encountered in two out of the five cases with *PTEN* mutations lacking mismatch repair deficiency or *POLE* mutations.

In our series, the ultramutated tumors seemed to have progressed to undifferentiated carcinomas by accumulating mutations in genes involved in the development of endometrioid carcinomas and/or *TP53* mutations. The dedifferentiated tumor with *POLE* mutation showed loss of ARID1A in both components but p53 overexpression and *TP53* mutation were found exclusively in the poorly differentiated/undifferentiated elements.[16] In contrast, the pure undifferentiated tumor had ARID1A loss and *PTEN* mutations. Our results are consistent with those of the TCGA, which reported mutations in *PTEN* (94%), *FBXW7* (82%), *ARID1A* (76%), *PIK3CA* (71%), and *TP53* (35%) in the *POLE*-mutated tumors.[19] Regarding prognosis, although the size of our series is not large enough to extract conclusions, we observed that the four patients classified as copy-number high or copy-number low to copy-number high had deceased, whereas the two patients classified as copy-number low were alive at the time of this study (no data were available for the third patient classified as copy-number low).

In our series, the study of the complete exome in one dedifferentiated carcinoma with rhabdoid-like features revealed the presence of a heterozygous mutation in *SMARCB1* limited to the undifferentiated component. This led us to analyze SMARCB1 (INI1) expression in the entire series; however, no tumor showed loss of SMARCB1 expression. Thus it appears that inactivation of one copy of *SMARCB1* is not enough for the development of rhabdoid features in undifferentiated carcinomas. However, other studies have demonstrated that expression of *SMARCB1* and more frequently of *SMARCA4* can be lost in the undifferentiated component of dedifferentiated carcinomas.[22,23] These findings, together with our observation of frequent ARID1A loss, suggest a role of SWI/SNF complex alterations in the pathogenesis of an undifferentiated phenotype in endometrial carcinomas.

In summary, this study shows that undifferentiated endometrial carcinomas may share molecular genetic alterations with any of the four molecular subgroups of endometrial cancer described by TCGA. Most undifferentiated carcinomas with a differentiated component (dedifferentiated carcinomas) occurred in the setting of mismatch repair deficiency (hypermutated tumors) with accumulation of molecular genetic alterations characteristic of endometrioid carcinomas, such as *ARID1A*, *PIK3CA* or *CTNNB1* mutations in the undifferentiated component. *TP53* mutation may act as the initial driver for some undifferentiated carcinomas developing through a 'serous-like' pathway or it may be involved in the progression of endometrioid tumors without microsatellite instability but exhibiting *PTEN* mutations. Finally, undifferentiated carcinomas carrying *POLE* mutations can evolve through endometrioid and/or 'serous-like' pathways.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on *Modern Pathology* website (http://www.nature.com/modpathol)