DICER and SMARCA4 mutations in ovarian neoplasia: recent developments.

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DICER1

Among sex cord stromal tumors, Sertoli-Leydig cell tumors are much less common than granulosa cell tumors and are typically divided in three categories, well, intermediate, and poorly differentiated. Furthermore, some tumors may show retiform or heterologous elements. Other tumors with "Sertoli" differentiation include Sertoli cell tumor and gynandroblastoma which are even less common than Sertoli-Leydig cell tumors. In recent years there has been important progress regarding the molecular bases of these tumors, especially through massive sequencing studies that have shown that somatic DICER1 mutations play an important role in these categories of tumors.

DICER1 gene encodes an endoribonuclease which has several functions but most notably is essential for control of gene expression through processing microRNAs. The latter are non-coding RNA molecules with functions including translation and degradation of messenger RNA, regulating expression of over 30% of protein coding genes at the post-transcriptional level. MicroRNAs are typically downregulated in cancer and may function as oncogenes or tumor suppressor genes.

The association of germline mutations in DICER1 (truncating mutations that spread across the gene) in patients with pleuropulmonary blastoma or the related familial tumor-dysplasia syndrome were first reported in 2009. The syndrome has an early childhood onset and it also includes cystic nephroma, ovarian sex cord stromal tumors (especially Sertoli-Leydig cell tumor -interestingly a small number bilateral-), multinodular goiter and other much less common tumors (cervical embryonal rhabdomyosarcoma, nasal chondromesenchymal hamartoma, ciliary-body medulloepithelioma, pituitary blastoma, juvenile intestinal hamartomatous polyps, and Wilms tumor). The penetrance of phenotypes is not known. Overall, female carriers seem to be more likely affected, but a large number of mutation carriers are clinically well. It has been suggested that tumors related to the DICER syndrome possess an embryonal or primitive appearance. In particular, one recent study on sex cord stromal tumors in patients with germline DICER1 mutations has shown that tumors are characterized by marked architectural and cytologic heterogeneity including sertoliform, juvenile granulosa cell and unclassifiable areas although all mostly resembled Sertoli-Leydig cell tumor, intermediate or poorly differentiated. The tumors were FOXL2 positive by immunohistochemistry without associated mutations.

However, the Dicer gene has other functions/alterations:

a) Dicer levels have global effects on miRNA biogenesis, with reduced expression being correlated to poor outcome in cancer.
b) Dicer function appears to be tissue specific in development and differentiation. In mouse models with urogenital-specific knockout of Dicer1 there is evidence of apoptosis of germ cells and Sertoli cells. It has been shown that somatic hotspot mutations (typically involving exons 24 and 25) occur most commonly in Sertoli-Leydig cell tumors. In contrast, they are lacking in sex cord stromal tumors of the testis including typical Sertoli cell tumors, large calcifying Sertoli cell tumors and sex cord stromal tumors not otherwise specified, although the number of neoplasms tested in this category is very limited. In fact, Dicer has been shown to have an important role in fertility in both the ovary and the testis. In the ovary it is expressed in oocytes and granulosa cells and reduced Dicer levels is associated with reduced ovulation rate secondary to compromised folliculogenesis. Even though in the testis Dicer is also very important in the development of Sertoli cells and spermatogenesis, in contrast to sex cord stromal tumors of the ovary with “Sertoli” differentiation, no somatic mutations are noted in the sex cord stromal tumors of the testis, potentially indicating that the pathogenesis of these tumors in the ovary and testis differs.

Somatic hotspot mutations, which are typically missense mutations, have been identified in approximately 60% of ovarian Sertoli-Leydig cell tumors, 40% of ovarian Sertoli cell tumors (including one lipid rich) and gynandroblastomas, and more rarely in juvenile granulosa cell tumors, unclassified sex cord stromal tumors and other tumor types including embryonal rhabdomyosarcoma in cervix and rhabdomyosarcoma arising in a malignant mixed müllerian tumor, and ovarian and testicular germ cell tumors (yolk sac tumor, gonadoblastoma/dysgerminoma, and teratoma).

When specifically looking at Sertoli-Leydig cell tumors, it has been shown that mutations are seen with similar frequency in intermediate and poorly differentiated neoplasms but do not occur in well differentiated tumors, although only a small number in the latter category have been tested. The finding of hotspot mutations is not related to the presence of retiform morphology or heterologous elements including rhabdomyosarcoma. The most frequent hotspot mutations reported include p.E1705K (c.5113G>A) and p.D1709N (c.5125G>A), seen in 80% of tumors. In one study, an identical mutation was noted in areas of conventional Sertoli-Leydig cell tumor and in areas of rhabdomyosarcoma, one case had the mutation only in the rhabdomyosarcoma component and in another case the mutation was noted in the retiform component but not in the rhabdomyosarcoma component. It has been recently reported that DICER1 mutant Sertoli-Leydig cell tumors have increased ER expression when compared to wild type DICER1 tumors. It has also been noted that a more aggressive behavior may be noted in patients with DICER1 mutated tumors.

Among wild-type DICER1 gynandroblastomas with material for FOXL2 study (2 out of 3), FOXL2 hotspot mutations were identified in both tumors, in granulosa and Sertoli cell areas. Even though, results may suggest that two genetic categories of gynandroblastoma exist as the two mutations appear to be mutually exclusive in ovarian sex cord stromal tumors, no differences are appreciated between the two groups on morphologic grounds. Of interest, very recently DICER1 hotspot mutations have been reported in 2 juvenile granulosa cell tumors.
It has been shown that the acquisition of somatic *DICER1* mutations is a very rare event in carcinomas, and if present is typically seen in microsatellite instable carcinomas and thus it does not represent a drive mutation.

Although *DICER1* is a frequent genomic event across the spectrum of ovarian sex cord tumors with "sertoliform" differentiation. It is important to keep in mind that *DICER1* mutated tumors cannot be distinguished from *DICER1* wild type tumors based on morphology. Testing for *DICER1* hotspot mutations in poorly differentiated tumors or those that are about to be classified as sex cord stromal tumor unclassified may narrow down the diagnosis to sex cord stromal tumor within the category of tumors with Sertoli differentiation.

**SMARCA4**

Small cell carcinoma, hypercalcemic type, is an uncommon but highly aggressive ovarian tumor that occurs in young females. It is associated with hypercalcemia in approximately 60% of cases. Its morphology is quite characteristic and includes small cells with brisk mitotic activity that grow in sheets, nests or cords and may show follicle-like spaces. In approximately 50% of the tumors a component of large cells, in some instances with a rhabdoid morphology, can be seen. Although the morphology is characteristic, its rarity often causes diagnostic problems with other more common ovarian tumors.

SMARCA4 is part of the SWI/SNF complex (which also includes SMARCB1, ARID1A, and ARID1B) and regulates gene expression by remodeling chromatin. It is estimated that mutations in SWI/SNF components occur in up to 20% of tumors with SMARCA4 mutations being among the most common. Recurrent SMARCA4 mutations (which are destructive of the protein-truncating, frameshift, splice site, or deletions with common bi-allelic inactivation) resulting in loss of SMARCA4 protein occur in 90% small cell carcinoma of hypercalcemic type indicating that SMARCA4 loss is highly sensitive and very specific for this tumor type. Thus SMARCA4 immunohistochemistry can be used as a surrogate marker and applied when problems in its differential diagnosis. However, one potential pitfall is preserved staining for SMARCA4 despite SMARCA4 mutations which has been reported in at least one case. It is important to keep in mind that rarely clear cell carcinoma of the ovary can show SMARCA4 loss (typically missense mutations which do not involve inactivation of the second allele). The morphologic appearance of these tumors does not overlap with the small cell carcinoma of hypercalcemic type including the large cell variant, emphasizing that molecular or immunohistochemical findings should be correlated with morphologic features. Other tumors that may enter the differential diagnosis including small cell carcinoma of pulmonary type, granulosa cell tumors, Sertoli-Leydig cell tumors, and dysgerminoma typically retain SMARCA4 expression. Non-gynecologic tumors that may cause problems in differential diagnosis and may be centered in the ovary or peritoneum as desmoplastic small round cell tumor, embryonal rhabdomyosarcoma, melanoma or lymphoma also have retained SMARCA4 expression. In the uterus, endometrioid carcinoma, dedifferentiated carcinoma, and endometrial stromal sarcoma (either low or high grade, or rhabdoid morphology) may also display SMARCA4 loss.

Of interest SMARCA2 expression is concomitantly lost in small cell carcinoma of the hypercalcemic type (with only scattered tumor cells being positive; localization correlating with
morphology, nuclear in small cells and either nuclear or cytoplasmic in large cells) without associated mutations. In contrast, clear cell carcinoma typically shows loss of SMARCA4 or SMARCA2 but not both at the simultaneously. SMARCA2 can also be lost in dedifferentiated carcinoma and high-grade endometrial stromal sarcoma. It appears that the mechanism underlying the loss of protein is secondary to epigenetic silencing of the SMARCA2 gene. This concomitant loss of SMARCA4 and SMARCA2 has been noted in lung tumors associated with poor prognosis.

The function of the SWI/SNF complex in transcriptional regulation, the absence of other recurrent mutations and the diploid cytogenetic profile of small cell carcinoma of hypercalcemic type indicates as main mechanism of oncogenesis epigenetic dysregulation and the poorly differentiated nature of the tumor as well as its highly proliferative index suggests failure to activate genes that promote or maintain terminal differentiation. In parallel to small cell carcinoma of hypercalcemic type, rhabdoid tumors also show deficiency of SMARCA1 by mutation and SMARCA2 by non-mutational silencing. These two tumor types share a lethal behavior, diploid cytogenetics, small cell/rhabdoid histology and dual loss of core SWI/SNF components that has lead the hypothesis that small cell carcinoma of hypercalcemic type is a malignant rhabdoid tumor, however, the change in nomenclature may be too premature as at least by morphology, only a small number of tumors in this tumor type show rhabdoid cells and may be a more appropriate term may be SMARCA4 mutated phenotype keeping the original name applied by Dr Robert E Scully, who first recognized and characterized this tumor.

REFERENCES:


