Lavage of the uterine cavity as potential tool for diagnosis of epithelial ovarian cancer and its precursors

Gynecologic Cancer Intergroup
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Type II serous pelvic cancer - Typ II ovarian cancer

HGSC: type II
- fast growing, disseminating tumors
- >95% show p53 inactivation
- frequent inactivation of BRCA1/2 DNA damage repair pathway

LGSC: type I
- slow transition from benign to malign
- somatic mutations in KRAS, BRAF..
Ovarian cancer development from fallopian tubes

- Ovaries generate a unfavorable microenvironment, by releasing toxic substances after ovulation
- Creates local inflammatory processes affecting the epithelium of fallopian tube fimbrae
- Leading to neoplastic changes
- Malignant transformation

Correlation between expression patterns of HGSC and fallopian tube epithelium in whole genome expression profiling (PAX8,..)
p53 Signatures – earliest step in pathogenesis of tubal carcinoma

- Cytological benign tubal epithelium (secretory cells, not ciliated)
- Immunohistochemistry: p53 positive nuclei, Ki-67 low, reduced number of p21, p27 positive cells
- TP53 mutations 50%
- $\gamma$H2AX expression as sign of DNA damage
- Common in BRCA mutation carriers, but also found in healthy women
- P53 inactivation is necessary but not sufficient for transformation
Epithelium containing small secretory cell-rich linear segments
STICs – Serous tubal intraepithelial carcinomas

- No definitive precursor of HGSC in the ovary
- Identified through histological examination of tissue from prophylactic salpingo-oophorectomy
- Present in up to 10% of prophylactic oophorectomies
- Presence of STICs in the absence of HGSC
- Identical TP53 mutations of STICS and HGSCs
- Analysis of tissue from sporadic cases of HGSC – STICs present in fallopian tube in 67% of cases, 92% of STICs confined to the fimbriated end
- Non invasive carcinomas, further genetic changes and/or alterations in cellular phenotype must occur prior transition to HGSC
- Immunohistochemistry: p53 positive, Ki-67 positive
• **LUDOC:** Lavage of the uterine cavity for the Diagnosis of Ovarian, tubal & peritoneal Carcinoma
  ▪ Patients with suspicion for serous pelvic cancer

• **LUSTIC:** Lavage of the Uterine cavity for the diagnosis of Serous Tubal Intraepithelial Carcinomas
  ▪ BRCA 1/2 mutation carriers,
  ▪ rrBSO (risk reducing bilateral salpingo-oophorectomy)
The ALPINE technique – (Austrian Lavage Procedure for the Detection of tubal Intraepithelial Neoplasms)
Use of routinely collected DNA from liquid Pap for somatic mutation detection, present in tumor cells that accumulate in the cervix.

- Panel of commonly mutated genes in endometrial/ovarian cancer, obtained from whole-exome sequencing data.

- Tumor tissue of 24 endometrial, 22 ovarian cancers
100% (24 of 24) Endometrial cancers
41% (9 of 22) Ovarian cancers
positive for the respective mutation detected in tumor tissue
Uterine lavage, matched tumor tissue

Digital droplet PCR (ddPCR)
MUW Vienna, R. Zeillinger
KRAS, TP53

Massively parallel sequencing (Illumina)
John Hopkins Univ., Baltimore, B. Vogelstein
AKT1, APC, ARID1A, BRCA, BRCA2, BRAF, CTNNB1, CSMD3, CDKN2A, EGFR, FBXW7, FAT3, FGFR2, KRAS, MLL2, NRAS, PTEN, PIK3CA, PIK3R1, PPP2R1A, PIK3R, RNF43, and TP53

Johns Hopkins Kimmel Cancer Center, The Ludwig Center for Cancer Genetics and Therapeutics, Bert Vogelstein - Baltimore, USA

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Digital droplet PCR (ddPCR) – n=9

8 patients with ovarian cancer ➔ 7/8 (87.5%)

Additionally: 1 signet ring carcinoma with ovarian metastases
23 patients with ovarian cancer $\Rightarrow 17/23$ (74%)

additional: 1 signet ring carcinoma with ovarian metastases
1 borderline tumor

0.15% - 46.2% mutation rate
Illumina Sequencing vs. ddPCR

R² = 0.9915
Immucytochemical detection of cancer cells in the lavage through 8µM membrane filtration and TP53 staining.
Current project - LUDOC

50 cases of uterine lavages & liquid PAP

Massively parallel sequencing (Illumina)

AKT1, APC, ARID1A, BRCA, BRCA2, BRAF, CTNNB1, CSMD3, CDKN2A, EGFR, FBXW7, FAT3, FGFR2, KRAS, MLL2, NRAS, PTEN, PIK3CA, PIK3R1, PPP2R1A, PIK3R, RNF43, and TP53
- Lavage of 200 BRCA mutation carriers undergoing rrBSO
- Extensive examination of tissue
  - Morphologic criteria,
  - Immunohistochemistry - p53, Ki-67, laminin γ1 (p53 null mutations)
  - Micro-dissection
  - Sanger sequencing - TP53 mutation status
- Processing of Lavage specimen
  - TP53 mutational analysis using ddPCR (TP53 status from matched tissue)
  - Immunocytochemistry: p53, Ki-67, p73 (discrimination ciliated/secretory cells)
Future projects – prospective evaluation differential diagnosis

Women with adnexal masses – IOTA, Berlin Study

Future projects – pilot study endometrial pathologies

Women undergoing EM sampling D&C – sup. EM pathologies

Future activities – international working group for the study of STICs

EUTROC, ENGOT, GCIG, …
Different material – supernatant, other DNA markers, …
Other targets - L1, telomeric length, …
Other techniques – immunocytochemistry, …
…
Clinical Partners

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