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Correlations between *c-myc* gene copy-number and clinicopathological parameters of ovarian tumours

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ABSTRACT

The objective of this study was to investigate increases in *c-myc* gene copy-number in ovarian tumours, and to analyze their correlations with clinicopathological parameters. Here we applied FISH on TMA (tissue microarrays) containing 507 ovarian tumour samples from different malignancy, histology, stage and grade. Overall, we found high frequency for *c-myc* copy-number increases (38.5%) in ovarian cancers: 22.1% amplifications and 16.4% gains. We established *c-myc* amplification in more than 30% in endometrioid and mixed epithelial ovarian carcinomas. *c-myc* gains were found in a high proportion (42.9%) of clear cell carcinomas. We found associations between *c-myc* copy-number changes and clinicopathological parameters of ovarian tumours such as degree of malignancy and histological type. We suggested that *c-myc* amplifications are characteristics for endometrioid, and *c-myc* gains for clear cell ovarian cancers. We suggest that copy-number increases of *c-myc* and 20q13.2 represent a possible mechanism for the regulation of the pathway *STK15* – *c-myc* – hTERT.

1. Introduction

Ovarian cancer, although second in incidence as a gynaecologic cancer, causes more deaths than all other gynaecologic cancers combined. This tumour type represents 4% of all cancer cases. It is tied with pancreatic cancer as the fourth most common cause of cancer death in women, being preceded by cancer of the lung, breast and colon and rectum.[1] Unfortunately, the pathogenesis of this disease is poorly understood, but a deeper knowledge of the biology of ovarian cancer would be the base for the development of new therapeutic concepts. The understanding of the molecular pathogenesis of ovarian cancer has been hindered by the lack of sufficient numbers of specimens at early stage disease because of its frequent diagnosis at an advanced stage.

As in many other cancers, the initiation and progression of this cancer involve accumulation of genetic changes, such as rearrangements, amplifications, and deletions affecting critical genes for cell growth, differentiation and death.[2] Ovarian carcinomas show complex cytogenetic rearrangements.[3] Bayani and colleagues investigated a set of ovarian cancers and identified by spectral karyotyping, that chromosomes 3, 8, 11, 17 and 21 had the highest frequencies of structural and numerical aberrations.[4] The most frequently affected chromosomal regions in ovarian tumours, detected by comparative genomic hybridization (CGH), are 3q, 8q and 20q, often with high-level amplification.[5] Genes, activated by such chromosomal alterations may be primary mediators of the clonal progression of cancer. Comparative genomic hybridization has determined the copy-number increases of the region

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8q24 as very common in ovarian tumours (35–76%).[6–15] This region contains the gene *c-myc* for transcription factor, which was found to cause the formation of murine ovarian tumours that were similar to human ovarian carcinomas.[16]

c-myc protein binds to the promoters of at least five genes. It is a transcription activator of proliferation factors, and also an inhibitor of factors suppressing growth.[17] *c-myc* induces the transcription of the genes *e2f1*, *e2f2* и *e2f3* and blocks the function of p53 by binding the promoter of *p21*.[18] Finally, there is evidence for direct activation of telomerase activity by c-myc, and is known to be a key activator of human telomerase reverse transcriptase (hTERT) transcription.[19] Moreover, c-myc appears to be mediator of telomerase activation by Aurora-A kinase gene (*STK15*), the key gene of 20q13.2 amplicon, as the later simultaneously induces c-myc expression and telomerase activity in a dose-dependent manner.[20] *c-myc* is targeted in vitro as anti-oncogene therapy by specific oligonucleotides.[21,22] This pinpoints *c-myc* as critical cancer-emerging gene.

Tissue microarray (TMA) technology enables us to perform molecular analysis in large numbers of tumour samples, leading to discoveries that underpin the clinicopathological significance of gene copy-number alterations. Here, we have applied fluorescent in situ hybridization (FISH) of *c-myc* on TMA, containing large number of ovarian tumours from different malignancy, histology, tumour stage and histological grade in order to establish the frequency of *c-myc* copy-number alterations in different ovarian tumours, to evaluate the genetic heterogeneity of ovarian tumours, to analyze the correlations of *c-myc* copy-number changes with clinicopathological parameters, and to compare the *c-myc* copy-number changes with other related gene copy-number alterations.

2. Materials and methods

2.1. Ovarian tumours tissue microarray (TMA)

TMA containing 503 ovarian tumour samples from formalin-fixed paraffin-embedded blocks of 507 patients was constructed. The blocks were collected from the gynaecopathological department of University Hospital of Obstetrics and Gynaecology, Sofia. There were 234 malignant, 24 low malignant potential and 245 benign tumours. Ovarian tumours vary considerably in their histological type. TMA contained specimens from all histological variants: 222 serous, 67 mucinous, 72 endometrioid, 8 clear cell, 5 Brenner, 25 mixed epithelial, 26 non-differentiated, 21 non-classified, 25 granulose-cell, 36 germline-cell tumours (teratomas, dysgerminomas). Histological heterogeneity may be associated with genetic heterogeneity. The haematoxillin-eosin (H&E) stained slides from all tumours were reviewed prior to construction by a single pathologist and representative areas of each tumour were determined. Tumour stage and grade were defined according to FIGO and WHO criteria.[23]

Sections of the microarray provide targets for parallel in situ detection of DNA, RNA and protein targets in each specimen on the array, and consecutive sections allow the rapid analysis of hundreds of molecular markers in the same set of specimens. For TMA construction, a hematoxilin and eosin (H&E)-stained section was made from each block to define representative tumour areas. Tissue cylinders with a diameter of 0.6 mm were punched from tumour areas and brought into a recipient paraffin block using a custom-made precision instrument.[24] Samples were distributed in one regular-sized recipient paraffin block containing 507 specimens. Five micrometer sections of the blocks were transferred to glass slides using a paraffin-sectioning aid system (adhesive coated slides, adhesive tape, UV-lamp; Instrumedics Inc., Hackensack, NJ).

2.2. Fluorescent in situ hybridization (FISH)

Fluorescent in situ hybridization is a rapid method, highly specific and sensitive for evaluation of particular genetic aberration. It represents direct visualization of fluorescence labeled DNA sequence on interphase or metaphase nuclei. Here we used FISH for detection of copy-number of *c-myc*. Prior to hybridization, the slides were treated with Paraffin Pretreatment Reagent Kit (Vysis). FISH was performed using a locus-specific probe for *c-myc* labeled in Sectrum Orange (Vysis, Cat #30-190006). Denaturation of the DNA was carried out at 75 °C for 10 min (probe mixture) or 5 min (slides). The probe mixture was applied to the slides and hybridized overnight in a moist chamber at 37 °C. The post-hybridization washes were performed as described in "LSI procedure" (Vysis). Slides were counterstained with DAPI in anti-fade. The presence of >4 copies per cell or tight clusters in at least 10% of tumour cells was considered as amplification according to the instruction of Vysis (Pathvysion). Presence of more than two but $\leqslant$4 gene signals in at least 10% of tumour cells was considered a "gain".

2.3. Statistical analysis

The relationship between copy-number changes and clinicopathological data was estimated using χ^2 test and *P*-value was calculated. $P < 0.05$ was required for significance.

3. Results

3.1. c-myc copy-number changes

A TMA including 507 ovarian tumour samples was analyzed by FISH for *c-myc* copy-number changes (amplifications and gains). FISH was successful in 75% of the tumours (380 samples – 280 malignant, 23 low malignant potency and 77 benign tumours). Copy-number changes of *c-myc* were found in 38.5% of all ovarian malignancies, in 26.1% of tumours with low malignant potential and in 7.8% of benign ovarian tumours (Table 1). These alterations were associated with the degree of malignancy ($P < 0.0001$).

3.2. c-myc amplifications and malignancy

c-myc amplification was strongly associated with the degree of malignancy of ovarian tumours, the frequency of this alteration increased statistically from low malignant potential (8.7%) to malignant (22.1%) tumours and was 0 in benign adenomas ($P < 0.0001$) (Table 1).