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Expression, activation and clinical relevance of CHK1 and CHK2 in metastatic high-grade serous carcinoma☆

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HIGHLIGHTS

- CHK1 and CHK2 are overexpressed in post-chemotherapy HGSC effusions compared to pre-chemotherapy specimens.
- CHK protein expression and activation is associated with poor survival in metastatic HGSC
- CHK1 and CHK2 may be candidates for targeted therapy in this cancer, particularly in recurrent disease.

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ABSTRACT

Objective. To analyze the expression and clinical role of CHK1 and CHK2 in metastatic high-grade serous carcinoma (HGSC).

Methods. HGSC effusions (n = 335; 280 peritoneal, 55 pleural) were analyzed for protein expression of total CHK1 and its phosphorylated forms p-ser317 and p-ser296, as well as total CHK2 and its phosphorylated form p-thr68 using immunohistochemistry. Expression was analyzed for association with clinicopathologic parameters, including chemotherapy response, and survival.

Results. Carcinoma cells stained positive, predominantly at the nuclei, in the majority of cases (range 83–100% for the five antibodies), while expression in reactive mesothelial cells and tumor-associated macrophages was more variable. Total CHK1 ($p = 0.037$), p-CHK1ser317 ($p = 0.001$), p-CHK1ser296 ($p = 0.002$) and p-CHK2thr68 ($p < 0.001$) expression was significantly higher in post-chemotherapy disease recurrence compared to pre-chemotherapy effusions obtained at diagnosis. CHK1, p-CHK1ser296, p-CHK2thr68 and p-CHK1ser317 nuclear expression was positively related to expression of the checkpoint regulator WEE1, previously studied in this cohort ($p = 0.003$, $p = 0.013$, $p = 0.001$ and $p = 0.01$, respectively). Higher total CHK1 ($p = 0.007$), p-CHK1ser317 ($p = 0.004$), CHK2 ($p = 0.01$) and p-CHK2thr68 ($p = 0.048$) expression was significantly related to shorter overall survival in univariate analysis, and CHK1ser317 was an independent prognostic marker in multivariate analysis ($p = 0.025$). Higher p-CHK1ser317 ($p = 0.03$) and CHK2 ($p = 0.034$) expression was additionally associated with poor progression-free survival.

Conclusions. CHK1 and CHK2 and their activated forms are frequently expressed in HGSC effusions, with higher expression following exposure to chemotherapy, and their expression is related to survival.

1. Introduction

The preservation of genomic integrity is essential for cells and organisms. DNA damage occurs repeatedly in mammalian cells due to both exogenous and endogenous causes and triggers the DNA damage response (DDR), which combines DNA repair with temporary cell cycle arrest, thus preventing deleterious effects. Extreme damage to the DNA results in cell death. Cancer cells are characterized by genomic

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instability and extensive DNA alterations, which are further augmented by radiation and chemotherapy.

Cell cycle arrest or delay may occur at 3 major checkpoints, i.e. G1/S, intra-S and G2/M. p53 has a central role in controlling the G1/S checkpoint, and its loss or deactivation, occurring in the majority of cancers, forces cancer cells to rely on the S and G2/M checkpoints for repair following DNA damage [1,2].

The Ataxia Telangiectasia Mutated (ATM) kinase is activated by DNA double-strand breaks. ATM activates the G1 checkpoint and prevents cells from progressing to the S- phase by phosphorylating and activating checkpoint kinase 2 (CHK2), which in turn inhibits CDC25A, the phosphatase that removes the inhibitory phosphorylation of cyclin-dependent kinase 2 (CDK2), part of the cyclin A/CDK2 and cyclin E/CDK2 complexes.

The Ataxia Telangiectasia and Rad3-related (ATR) kinase is activated when single-strand DNA structures are generated at stalled replication forks, during nucleotide excision/repair or from resected DNA double-strand breaks. ATR induces transient cell cycle arrest and DNA repair, mediated by its downstream target CHK1. ATR and CHK1 additionally activate the G2/M checkpoint, preventing cells from entering mitosis. The latter occurs via ATR-mediated inhibition of cyclin B/CDK1 through activation of its inhibitor, WEE1 kinase, as well as by CDC25C inhibition via CHK1 [1,2].

The dependence of cancer cells on DDR has generated considerable interest in targeting molecules regulating this process, including ATM, ATR, WEE1 and CHK, with the aim of pushing tumor cells into mitotic catastrophe [1–6].

Ovarian cancer, consisting mainly of ovarian carcinoma, is the most lethal gynecological malignancy. Recent advances in surgery, optimization of chemotherapy protocols, and the use of targeted therapy have led to improvement in overall survival. However, the majority of ovarian cancer patients eventually die of the disease, mainly due to late diagnosis and intrinsic or acquired chemoresistance [7]. Thus, identifying novel biomarkers which may aid in predicting chemoresponse and survival, and may be therapeutically targeted, is highly relevant.

High-grade serous carcinoma (HGSC), the most common histotype of ovarian carcinoma, is characterized by gross genomic instability and mutations in genes regulating DNA repair, making it an ideal cancer for DDR inhibition. CHK status has been analyzed in several studies of this cancer, both in the experimental setting and in clinical specimens [8–16]. However, to the best of our knowledge, none of these reports had focused on metastatic HGSC in effusions. The present study analyzed the expression and clinical relevance of CHK1 and CHK2 in a large series of HGSC effusions.

2. Materials and methods

2.1. Patients and specimens

Effusion specimens (n = 335; 280 peritoneal, 55 pleural) from 335 patients diagnosed with HGSC were submitted for routine diagnostic purposes to the Department of Pathology at the Norwegian Radium Hospital during the period of 1998–2011. HGSC specimens and clinical data were obtained from the Department of Gynecologic Oncology, Norwegian Radium Hospital. This cohort included both patients who had upfront surgery and received adjuvant chemotherapy (n = 179) and patients who received neoadjuvant chemotherapy (n = 114). The remaining patients were inoperable and received only chemotherapy, received no therapy, or were operated at other hospitals, with no data regarding upfront treatment. Data regarding first-line chemotherapy were available for 324 of 331 patients who received chemotherapy, of whom 304 (94%) received platinum-based chemotherapy. Clinicopathologic data are detailed in Table 1.

Effusions were centrifuged immediately after tapping, and cell blocks were prepared using the Thrombin clot method. Effusions were diagnosed by an experienced cytopathologist (BD) based on morphology and immunohistochemistry (IHC). Immunostains applied at the Norwegian Radium Hospital for diagnosing tumors with serous morphology originating from the fallopian tube, ovary or peritoneum include PAX8, WT1 and p53. Other stains (e.g. Napsin A, HNF1β, PTEN, CEA and CDX2) are applied when tumors have non-serous morphology.

Table 1
Clinicopathologic data of the study cohort (335 patients).

Parameter		No. of patients
Age	Mean; range	63; 23–88
FIGO stage	I	2
	II	5
	III	193
	IV	132
	NA	3
CA 125 at diagnosis[a]	Range: 10–62,400	Median: 1296
Residual disease[b]	0	22
	≤1 cm	69
	>1 cm	84
	NA	4
Chemoresponse after primary treatment	CR	159
	PR	73
	SD	26
	PD	37
	NE[c]	40

Abbreviations: NA = not available; CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, NE = not evaluated.
[a] Available for 263 patients.
[b] For 179 patients with upfront surgery. Data for 114 patients who received neoadjuvant chemotherapy were as follows: RD = 0 cm: 25 patients; RD ≤1 cm: 46 patients; RD >1 cm: 31 patients; NA: 12 patients. Cases with no data include inoperable patients. The remaining 10 patients received only chemotherapy or had no data regarding upfront therapy.
[c] Disease response after chemotherapy could not be evaluated because of normalized CA 125 after primary surgery, missing CA 125 information and no residual tumor, or no data.

Informed consent was obtained according to national and institutional guidelines. Study approval was given by the Regional Committee for Medical Research Ethics in Norway (S-04300).

2.2. IHC

Formalin-fixed paraffin-embedded sections were analyzed for protein expression of total CHK1 and its phosphorylated forms p-ser317 and p-ser296, as well as total CHK2 and its phosphorylated form p-thr68 using the Dako EnVision™ Flex+ System (K8012; Dako, Glostrup, Denmark). Deparaffinization and unmasking of epitopes were carried out in a PT-Link (Dako) using an EnVision™ Flex target retrieval solution at high pH (Tris/EDTA pH 10). Sections were incubated with a 0.3% hydrogen peroxide (H_2O_2) solution for 5 min to block endogenous tissue peroxidase activity. Sections were then incubated with the relevant antibody. Antibody details and staining conditions are detailed in Supplementary Table 1. Sections were thereafter treated with EnVision™ Flex+ mouse or rabbit linker (15 min) and EnVision™ Flex/HRP enzyme (30 min), stained for 10 min with 3,3 diaminobenzidine tetrahydrochloride (DAB), counterstained with hematoxylin, dehydrated and mounted in Richard-Allan Scientific Cyto seal XYL (Thermo Fisher Scientific). Positive controls for the three CHK1 antibodies and for p-CHK2thr68 consisted of normal testis, whereas normal tonsil was used for CHK2. Negative controls were stained with non-relevant antibody of the same isotype for monoclonal antibodies and normal rabbit serum for polyclonal antibodies.

2.2.1. IHC scoring

Nuclear and cytoplasmic staining was considered positive. Staining extent was scored by two of the authors (BD and MB), the former experienced cytopathologist, using a 0–4 scale as follows: 0 = no staining, 1 = 1–5%, 2 = 6–25%, 3 = 26–75%, 4 = 76–100% of tumor cells. For antibodies staining tumor cell nuclei with variable intensity, expression was further scored as absent (=0), weak (=1) or strong (=2). For these values, a staining score was calculated based on multiplying the

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