



**Karolinska  
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**Institutionen för Onkologi-Patologi**

# Molecular steps towards improving prognosis in ovarian cancer

**AKADEMISK AVHANDLING**

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av

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## ABSTRACT

Epithelial ovarian cancer (EOC) is the most lethal of gynecological cancers, and cure rates have improved very little in the last 30 years. The most common histological subtypes are serous, endometrioid, clear cell and mucinous EOC. To date, all EOC have been treated as one entity. However, based on epidemiological and molecular studies it is now clear that the different subtypes should be considered as different diseases. Also, low-grade serous and high-grade serous EOC (HGSOC) has distinctive molecular characteristics. The majority of EOCs are HGSOCs characterized by genetic instability, advanced stage at presentation and acquired chemoresistance. There is an urgent need to identify new targets in order to improve prognosis for these tumors.

A deregulated energy metabolism is a hallmark of malignant disease that offers possible future targets for treatment. Its major features are an increased aerobic glycolysis and alterations in mitochondrial bioenergetics. This thesis aims at identifying prognostic and treatment predictive markers in advanced HGSOC. We specifically explore the expression of metabolic enzymes and heat shock protein 60 (HSP60) and test the chemo-potentiating effect of glycolysis inhibitor 2-deoxy-D-glucose (2-DG) *in vitro*.

We found a platinum-potentiating effect of 2-DG in two EOC cell lines and 17 freshly isolated ascites EOC samples. We also found the mitochondrial  $\beta$ -F1-ATPase:HSP60 ratio to be predictive of sensitivity to such combination treatment.

We prospectively collected fresh tumor samples from 123 patients undergoing primary surgery for advanced EOC. Of these, 56 met the eligibility criteria with adequate sample RNA yield. Ninety-three percent were high-grade tumors. We performed real-time PCR and immunohistochemistry to study the expression of HSP60, glyceraldehyde-3P-dehydrogenase (GAPDH), pyruvate kinase M2 (PKM2), mitochondrial  $\beta$ -F1-ATPase (ATP5B) and the bioenergetic cellular (BEC)-index. We used Cox proportional hazards models to estimate overall survival (OS) and platinum-free interval (PFI). A high HSP60 mRNA was associated with shorter OS (HR, 3.4 95% CI 1.3-8.5) and PFI (HR, 3.3; 95% CI 1.5-7.2). At the protein level, HSP60 was also of independent prognostic value, with a median survival difference of 24 months between high- and low expressing groups. All patients with low tumor HSP60 protein expression responded to primary chemotherapy. High GAPDH mRNA levels (HR 2.1, 95% CI 1.0-4.5) and low BEC-index mRNA (HR 0.47, 95% CI 0.23-0.95) were both independently associated with shorter PFI.

We also compared the mRNA expression of metabolic markers and HSP60 in a series of 25 matched serous solid tumors and corresponding detached tumor cells in ascites. GAPDH, PKM2, ATP5B and HSP60 did not significantly differ in these respective cell states, indicating that further reprogramming of glycolysis or oxidative phosphorylation is not a prerequisite for serous cancer cell survival after detachment.

This thesis validates targeting glucose metabolism for increasing treatment efficacy in EOC. Our findings also indicate that HSP60, GAPDH and BEC-index may, within the seemingly homogenous group of advanced HGSOCs, identify patients with different prognosis.