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Original contribution

HUR mRNA expression in ovarian high-grade serous carcinoma effusions is associated with poor survival☆

Ben Davidson MD, PhD[a,b,*], Arild Holth BSc[a], Ellen Hellesylt MSc[a], Rivka Hadar MSc[c], Betina Katz MD, PhD[a], Claes G. Tropé MD, PhD[b,d], Reuven Reich PhD[c,,1]**

[a]*Department of Pathology, Oslo University Hospital, Norwegian Radium Hospital, N-0310 Oslo, Norway*
[b]*University of Oslo, Faculty of Medicine, Institute of Clinical Medicine, N-0316 Oslo, Norway*
[c]*Institute of Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel*
[d]*Department of Gynecologic Oncology, Oslo University Hospital, Norwegian Radium Hospital, N-0310 Oslo, Norway*

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Summary The objective of this study was to analyze the expression and clinical role of the RNA-binding molecule HuR in metastatic high-grade ovarian serous carcinoma (HGSC). *HUR* mRNA expression by reverse-transcription polymerase chain reaction was analyzed in 66 effusions from patients diagnosed with HGSC. Protein expression was analyzed in 262 HGSC effusions using immunohistochemistry. *HUR* mRNA was detected in all 66 effusions. *HUR* mRNA levels were unrelated to clinicopathological parameters. However, higher *HUR* mRNA levels were significantly related to poor overall survival in the entire cohort ($P = .023$), as well as in analysis limited to patients with prechemotherapy primary diagnosis specimens ($P = .001$) in univariate analysis. Cox multivariate analysis showed an independent prognostic role for *HUR* mRNA in the entire cohort ($P = .033$) and in patients with prechemotherapy primary diagnosis specimens ($P = .002$). HuR protein was detected in the nucleus and cytoplasm of tumor cells in 258 (98%) of 262 and 153 (58%) of 262 effusions, respectively. Higher HuR protein expression was associated with higher serum Cancer Antigen (CA) 125 levels at diagnosis ($P = .01$), but its presence at both cellular compartments was otherwise unrelated to clinicopathological parameters or survival. In conclusion, HuR is widely expressed in metastatic HGSC at both the mRNA and protein level. Higher *HUR* mRNA levels are associated with poor survival in metastatic HGSC, whereas protein expression has no prognostic value.

1. Introduction

Ovarian cancer, predominantly consisting of ovarian carcinoma (OC), is the most aggressive gynecologic malignancy. OC is diagnosed at advanced stage, that is, International Federation of Gynecology and Obstetrics (FIGO) stage III/IV, in the majority of cases, and despite optimization of surgery and chemotherapy protocols the majority of patients succumb to the disease within 5 years

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* Correspondence to: B. Davidson, Department of Pathology, Norwegian Radium Hospital, Oslo University Hospital, Montebello, N-0310 Oslo, Norway.
** Correspondence to: R. Reich, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel.
E-mail addresses: bend@medisin.uio.no (B. Davidson), reich@cc.huji.ac.il (R. Reich).
[1] R. R. is affiliated with the David R. Bloom Center for Pharmacy and the Adolf and Klara Brettler Center for Research in Molecular Pharmacology and Therapeutics at The Hebrew University of Jerusalem, Israel.

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[1]. One of the factors which limit our ability to optimize treatment of OC is incomplete understanding of disease heterogeneity, largely due to dearth of studies focusing on metastases.

OC, particularly high-grade serous carcinoma (HGSC), characteristically metastasizes within the peritoneal, and less frequently the pleural cavities, with the formation of malignant effusions in these anatomic spaces, and OC cells in effusions have been shown to have a unique genotypic and phenotypic profile [2,3].

RNA-binding proteins (RBP) have in recent years received growing attention as critical regulators of mRNA fate. The hundreds of RBP currently recognized form ribonucleoprotein complexes with nascent mRNAs, thereby controlling, together with miRNAs, their localization and targeting to translation or degradation. Through this, RBP regulate multiple physiological and pathological processes, the latter including cancer [4].

Hu-antigen R (HuR), aka, ELAV1 (embryonic lethal, abnormal vision, Drosophila)–like protein 1 (ELAVL1), is member of the ELAV/Hu family. Whereas expression of other ELAV/Hu family members, which include HuB, HuC, and HuD, is limited to the nervous system, the 32-kDa HuR is ubiquitously expressed. HuR contains 3 RNA recognition motifs, of which 2 (RRM-1 and RRM-2) bind AU-rich elements at the 3′-untranslated region of the mRNA, whereas the third (RRM-3) binds the poly(A) tail. HuR binding results in mRNA stabilization and indirectly promotes translation to protein. In resting cells, HuR is primarily localized to the nucleus, whereas in activated cells it translocates to the cytoplasm where it stabilizes messages and prevents their degradation. There appears to be interplay between HuR and miRNA regulation [4-6].

HuR modulators include a myriad of agents including physical factors (ultraviolet light, heat shock), cytokines (interleukin 1β, transforming growth factor β), hormones (Adrenocorticotropic hormone [ACTH], androgen, estradiol), drugs (tamoxifen, gemcitabine), viruses (human papillomavirus [HPV]), and miRNAs. HuR in turn regulates multiple mRNAs with recognized role in cancer biology, including those of various cyclins, p53, Bcl-2, WNT5A, vascular endothelial growth factor, insulin growth factor receptor 1, matrix metalloproteinase 9, hypoxia-induced factor 1α, interleukins, cyclooxygenase 2 (COX2), and estrogen receptor. Accordingly, the clinical role of HuR has been demonstrated in many cancers [5,6].

HuR has been the focus of several studies of OC [7-14]. In experimental models, HuR was shown to positively regulate ARHI (Ras homolog member I; DRAS3) [7], class III β-tubulin [8,9], and COX2 [10,11]. HuR was in turn shown to be positively regulated, together with class III β-tubulin, by miR-200c [9] and negatively regulated by miR-519 [12].

A prognostic role for HuR protein expression in OC has been reported in several studies [8-10,13,14]. However, these studies have focused exclusively on carcinomas localized to the ovary. The clinical role of HuR in metastatic OC cells in effusions is therefore unknown at present. The aim of the present study was to assess the clinical role of HuR at both the protein and mRNA level in a large cohort of patients with OC effusions, focusing exclusively on HGSC.

2. Materials and methods

2.1. Patients and specimens

The material analyzed using reverse-transcription polymerase chain reaction (RT-PCR) consisted of 66 effusions (49 peritoneal, 17 pleural) from 66 patients diagnosed with advanced-stage (FIGO stage III-IV) HGSCs. In view of the fact that the fallopian tubes have not been adequately assessed in this cohort, tumors are all referred to as OC. Specimens were submitted for routine diagnostic purposes to the Department of Pathology at the Norwegian Radium Hospital during the period of 1998 to 2004. OC specimens and clinical data were obtained from the Department of Gynecologic Oncology, Norwegian Radium Hospital. Clinicopathological data are detailed in Table 1. Informed consent was obtained according to national and institutional guidelines. Study approval was given by the Regional Committee for Medical Research Ethics in Norway.

All effusions were centrifuged immediately after tapping, and cell pellets were frozen at −70°C in equal amounts of RPMI 1640 medium (GIBCO-Invitrogen, Carlsbad, CA) containing 50% fetal calf serum (PAA Laboratories, Pasching, Austria) and 20% dimethylsulfoxide (Merck KGaA, Darmstadt, Germany). Effusions were diagnosed by an experienced cytopathologist (B. D.) using morphology and immunohistochemistry (IHC), based on evaluation of smears and cell blocks prepared using the thrombin clot method.

2.2. RT-PCR

Total RNA was extracted from the 66 OC effusions using TriZol reagent. One microgram of total RNA was used for first-strand cDNA synthesis followed by specific gene product amplification with One-Step RT-PCR Kit (Invitrogen, Burlington, ON). Primer sequences were as follows:

HUR: Forward: 5′-TCGCAGCTGTACCACTCGCCAG-3′
Reverse: 5′-CCAAACATCTGCCAGAGGATC-3′
GAPDH: Forward: 5′-AGCCGAGCCACATCGCT-3′
Reverse: 5′-TGGCAACAATATCCACTTTACCAGAGT-3′.

Cycle parameters for *HUR* were as follows: heating at 94°C for 3 minutes, denaturation at 95°C for 15 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 20 seconds, for 27 cycles. Cycle parameters for *GAPDH* were as follows: heating at 94°C for 5 minutes, denaturation at 94°C for 15 seconds, annealing at 60°C for 15 seconds,