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Short Communication

Molecular profiling in fresh tissue with high tumor cell content promotes enrichment for aggressive adenocarcinomas in cervix

Mari Kyllesø Halle[a,b,*], Camilla Krakstad[a,b], Hilde Engerud[a,b], Bjørn Bertelsen[c], Helga B. Salvesen[a,b]

[a] Department of Gynecology and Obstetrics, Haukeland University Hospital, Bergen, Norway
[b] Centre for Cancer Biomarkers, Department of Clinical Science, University of Bergen, Bergen, Norway
[c] Department of Pathology, Haukeland University Hospital, Bergen, Norway

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ABSTRACT

Many emerging tools for comprehensive molecular profiling of malignant lesions demand fresh frozen tissue with a high tumor purity. Often, a tumor epithelial content of at least 80% is recommended. This approach may lead to a systematic bias, and therefore we explore if this introduces a selection of cases with a certain phenotype in cervical cancer. Clinicopathologic data for a population-based cohort of 328 patients have been studied. Fresh frozen tumor specimens were available for 151 of these patients and investigated for epithelial tumor cell portion in hematoxylin-stained frozen sections by light microscopy. The estimated tumor purity in the samples was compared with FIGO stage, histopathologic characteristics and survival. High tumor purity was significantly more often found in squamous cell carcinoma (SCC) compared to adenocarcinoma (AC) ($P=0.03$). For the subgroup of AC ($n=40$), there was a significant association between high tumor purity in the fresh frozen samples and later occurrence of recurrent disease ($P=0.04$). In SCC, no significant associations between tumor purity and disease stage, grade or outcome were found. Apparently in line with this, grade was found to influence prognosis in AC, but not in SCC. Our findings suggest that selection of samples based on high tumor purity in fresh frozen tissue may introduce a selection bias toward aggressive disease for the subgroup of AC, but not for SCC of the cervix. Thus, the prevalence of potential molecular biomarkers identified in AC in particular should be validated in a population-based setting to further explore clinical relevance. Also, molecular biomarkers only prevalent in subgroups with low tumor purity may go undetected in sample collections enriched for high tumor purity.

Introduction

Cervical cancer annually accounts for 275,000 deaths worldwide, being the third leading cancer type among women [1]. Although extensive screening programs have decreased the incidence of cervical squamous cell carcinomas (SCC), the prevalence of cervical adenocarcinoma (AC) has recently increased [2]. It is well documented that ACs of the cervix have a higher recurrence rate and worse prognosis than SCCs [3]. Still, studies including molecular profiling of cervical carcinomas in general and ACs in particular are sparse [3]. We have recently reported data for comprehensive molecular profiling of cervical carcinomas that may be important to improve our understanding of potential targets for developing new therapy [4].

Several tools for comprehensive molecular profiling have been found to be helpful for increasing our understanding of the molecular alterations involved in the malignant transformation and progression. These methods include large-scale genome sequencing, genome-wide expression, methylation and copy-number studies, and systems biology approaches to analyze the data. These approaches are likely to be vital to develop and implement more effective strategies to deliver personalized cancer therapy [5]. Many of these powerful techniques demand fresh frozen tissue with little stromal contamination, and often at least 80% tumor epithelial content is applied as an inclusion criteria [6].

In our recent report on endometrial carcinomas, we found that this approach introduced a selection bias toward enrichment of aggressive tumors [7]. The importance of accurate tumor cell content estimation was thoroughly investigated in recent times [8,9]. However, the impact of selecting samples based on high tumor

* Corresponding author at: Department of Gynecology and Obstetrics, Haukeland University Hospital, 5021 Bergen, Norway. Tel.: +47 55 97 07 23; fax: +47 55 97 49 68.
E-mail address: Mari.Halle@k2.uib.no (M.K. Halle).

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purity has been studied less extensively. In the present study, we therefore explored if enriching for high tumor purity lesions introduces systematic selection biases toward inclusion of cases with aggressive phenotype in cervical cancers.

Materials and methods

Clinical and histopathologic data for a total of 328 patients with primary cervical carcinomas from a demographically well-defined area, treated at the Department of Obstetrics and Gynecology at Haukeland University Hospital in Bergen, Norway from 2001 through 2012, have been characterized in relation to treatment and follow-up. Haukeland University Hospital is a referral hospital for patients in Hordaland County in Western Norway, representing about 10% and a similar pattern for incidence and prognosis as the total Norwegian population (Cancer Registry of Norway, http://kreftregisteret.no).

All patients were staged according to the current International Federation of Gynecology and Obstetrics (FIGO) staging system. Stage I is carcinoma strictly confined to the cervix; Stage II extends beyond the cervix, but does not extend into the pelvic wall or to the lower third of vagina; Stage III extends into the pelvic sidewall and/or involves the lower third of the vagina and/or causes hydronephrosis and/or a non-functioning kidney; and Stage IV extends beyond the true pelvis or involves the mucosa of the bladder and/or rectum [10].

In parallel with formalin-fixed, paraffin-embedded (FFPE) tissue for routine histopathologic evaluation, fresh frozen tumor tissue was collected for research purpose from 151 consented patients and explored by frozen sections for purity of the malignant epithelial component. The proportion of neoplastic cells in the tumor specimens was estimated by the senior author (H.S.) by frozen section light microscopy. Statistical analyses were performed using SPSS version 21.0. Research permission was granted by the Norwegian Social Data Service (15501) and local Institutional Review Board (REKIII nr. 052.01).

Results

The estimated tumor cell content ranged from 10% to 100% with a median value of 80%. High tumor purity defined as 80% or higher was found in 86 cases, while 65 cases had less than 80% malignant epithelial component as evaluated in the frozen tissue sections. When comparing clinicopathologic features for patients with no fresh tissue available (only FFPE tissue) to the patient group with available fresh tissue collected for research purposes in addition to FFPE tissue for routine diagnostics, recurrent disease ($P = 0.02$) and FIGO stage IB and II ($P < 0.001$) were significantly more frequent in the patient cohort with fresh tissue available for potential molecular profiling (Table 1). High tumor purity was seen significantly more often in SCC than AC ($P = 0.03$, Table 1). Otherwise, there was no significant association between tumor purity and any of the other assessed clinical and histopathologic parameters such as age, menopausal status, FIGO stage, grade or recurrent disease when both histologic subtypes were analyzed together (Table 1). There was a tendency, although not statistically significant, to inferior recurrence-free survival for patients with tumor purity of 80% and higher compared to less than 80% ($P = 0.1$, Fig. 1A).

In subset analyses within histologic subtypes, however, ACs ($n = 40$) demonstrated a tendency to an association between high tumor purity and high grade ($P = 0.07$) and a significant association with later development of recurrent disease ($P = 0.04$, Table 2). In line with this, the tendency observed for the whole population was most distinct for the ACs, with a trend toward poorer recurrence-free survival for ACs with 80% or higher malignant epithelial component ($P = 0.1$, Fig. 1B). In the subset analysis of SCC, tumor purity did not associate with any of the clinicopathologic variables or outcome (Table 2 and Fig. 1C). Apparently also in line with this, grade significantly influenced prognosis for patients with ACs in the whole cohort ($P = 0.001$, Fig. 2B), but not for patients with SCCs (Fig. 2C).

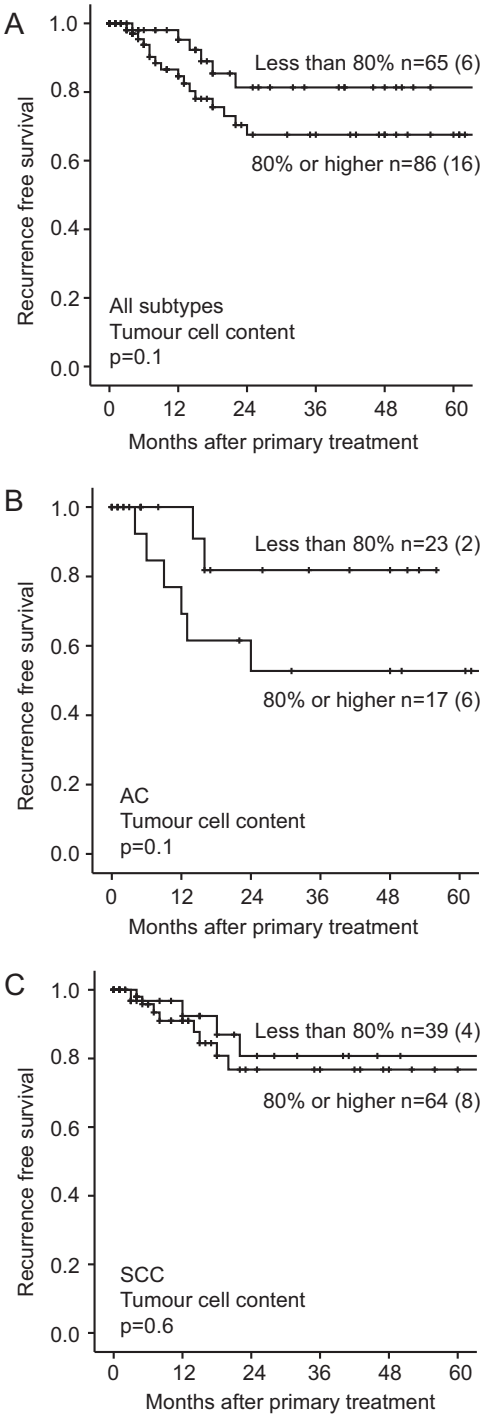

Fig. 1. Recurrence free survival for patients with cervical carcinoma related to tumor cell content for (A) all cases with fresh frozen tissue available, (B) adenocarcinoma (AC) and (C) squamous cell carcinoma (SCC). The number of patients, with number of events in parentheses, is given for each group. High tumor cell content, defined as 80% or higher, showed a tendency to association with poor recurrence free survival, most apparent for the subgroup of patients with adenocarcinoma. Kaplan–Meier survival curves are presented with *P*-values for Mantel–Cox log rank test comparing categories.