Editorial

# Identification of hypoxic gene-signature as a prognostic and predictive biomarker to determine effective therapy in high risk bladder cancer patients

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Bladder cancer is the ninth most commonly diagnosed cancer in the world and fifth in the USA and Canada (1,2). Patients presenting with bladder cancer are initially treated with transurethral resection and specimens are graded and staged. Muscle-invasive bladder cancers (MIBC) considered >pT2, are currently treated with radical cystectomy with neoadjuvant chemotherapy or a combination of radiation and chemotherapy (3-6).

Tumor hypoxia, which is common in multiple cancer types (7,8), is a biological condition that is characterized by deficient tissue oxygenation compromising biological functions. It interferes with the curability of solid tumors, regardless of the treatment modality employed (9). It is well-known that bladder cancer tumors, like other solid tumors, contain hypoxic areas and high expression of hypoxia-inducible markers have been associated with poor prognosis (10). There is good evidence that hypoxic tumors benefit from hypoxia-modifying therapy (11-13). However, there are no validated biomarkers that can select MIBC patients that would benefit from adding hypoxia-modifying therapy to radiotherapy. Although hypoxia gene signatures have been developed for head and neck, breast, and lung cancers, none have been developed for bladder cancer (12-14). In fact, the bladder carbogen and nicotinamide (BCON) phase III clinical trial showed that the addition of hypoxia-modifying therapy (carbogen and nicotinamide) to radiotherapy improved overall survival. Therefore, Yang and collaborators proposed to identify a hypoxia gene signature for MIBC patients, which predicted benefit from hypoxia-modifying therapy. To achieve this, the research group analyzed all the published gene signatures available in the literature. Contradictory to what has been published, Yang and collaborators found that only the Lendahl hypoxic signature (15) was predictive of benefit from hypoxic modification but not prognostic in MIBC, confirming the need to identify a hypoxia gene signature exclusive for MIBC patients. To derive the bladder cancer-specific hypoxia signature, Yang analyzed 611 generic hypoxia genes. He hypothesized that a candidate gene would likely be hypoxia regulated in the bladder if coexpressing with multiple candidate genes. Therefore, the group developed a bladder cancer-specific hypoxia gene coexpression network. The network comprised 168 candidate hypoxia genes with 458 significant interactions. They determined that the higher number of interactions between the candidate genes indicated the likelihood of hypoxia relevance in bladder cancer. In the end they found 24 highly expressed hypoxia genes which were significantly associated with poor prognosis. Comparisons of the 24-hypoxia gene signature with other two published hypoxia gene signatures showed a 4 gene overlap (*CAV1*, *P4HA2*, *DPYSL2*, *SLC2A3*) with Chi *et al.* study (16), and 2 gene overlap (SLC16A1 and LDLR) with the head and neck signature which previously had shown to be prognostic in multiple cancer types(14). The

authors discuss that the heterogeneity between signatures could be due to different biological pathways involved in tumor hypoxia in different cancer types, but also result from differences in the methods for obtaining these signatures.

Moreover, to investigate whether copy number variation biased the strength of gene-gene interactions, a null distribution was constructed to calculate the correlation values for 10,000 random gene-sets, of the same size as the gene pool. The correlations of the 24-gene signature were not significantly higher than the random sets (P=0.99), suggesting that the co-expression level was not driven by copy number variation. A similar analysis was performed to verify methylation data. It was found that the methylation rate of this 24-gene signature was significantly lower than that of random gene sets (P=0.0008), suggesting methylation status plays an important role in the computed co-expression level.

Furthermore, the *de novo* 24-gene signature was validated in several independent publically available cohorts comprising 679 fresh frozen tissue samples as well as in the BCON cohort of formalin-fixed paraffin embedded samples. Among BCON cohorts, protein expression data of three significant hypoxia biomarkers: carbonic anhydrase IX (CAIX), hypoxia-inducible factor 1-alpha (HIF-1α) and glucose transporter 1 (GLUT1) were analyzed and a *t*-test (two tailed) was performed. The data showed that the 24-gene signature score was significantly higher in tumors with high CAIX protein expression (P=0.013, upper quartile) and high HIF-1α protein expression (P=0.081, lower quartile). However, no significant association was detected between the signature score and GLUT1 expression. In order to assess the prognostic and predictive performance in a more complex system, they studied the role of CAIX, HIF-1α, GLUT1 and necrosis within the BCON cohorts. Their analysis confirmed that tumors with high CAIX expression had poorer LPFS with RT alone (n=64, P=0.022; HR, 2.21; 95% CI, 1.12–4.37) but benefited from CON (n=32, P=0.017; HR, 0.32; 95% CI, 0.13–0.82). High GLUT1 expression had no prognostic value in patients treated with only RT (P=0.20) while there was a predictive significance in patients treated with RT + CON (n=48, P=0.077; HR, 0.51; 95% CI, 0.24–1.08). There was no prognostic or predictive significance associate with HIF-1α expression. Necrosis was associated with poor prognosis (n=75, P=0.029; HR, 1.97; 95% CI, 1.08–3.60) and good predictive value (n=80, P=0.0051; HR, 0.43; 95% CI, 0.24–0.78) with a significant interaction (P=0.002). These assessments indicated that by combining necrosis and the gene signature score, a wider range of patients can be identified to benefit from hypoxia-modifying radiotherapy.

The current study not only successfully identified a de novo 24-gene signature in muscle invasive bladder cancer but also analyzed its correlation with other protein biomarkers to determine the prognostic and predictive value upon different tumor hypoxic levels. The authors also performed both multivariate and univariate analyses on the BCON cohort, evaluating any possible associations or interactions between each variable in bladder cancer progression (such as gender, age, tumor stage, etc.) and demonstrating no effects on the 24-gene signature. In conclusion, a hypoxia gene signature for muscle invasive bladder cancer patients was derived. The results from this study provide new clinical approaches to determine bladder cancer patients who would benefit from specific therapies including hypoxia modifying therapy. This study is an exemplary approach towards implementation of personalized medicine and integration of clinical studies into clinical practice.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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