CDX2 prognostic value in stage II/III resected colon cancer is related to CMS classification

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Background: Caudal-type homeobox transcription factor 2 (CDX2) is involved in colon cancer (CC) oncogenesis and has been proposed as a prognostic biomarker in patients with stage II or III CC.

Patients and methods: We analyzed CDX2 expression in a series of 469 CC typed for the new international consensus molecular subtype (CMS) classification, and we confirmed results in a series of 90 CC.

Results: Here, we show that lack of CDX2 expression is only present in the mesenchymal subgroup (CMS4) and in MSI-immune tumors (CMS1) and not in CMS2 and CMS3 colon cancer. Although CDX2 expression was a globally independent prognostic factor, loss of CDX2 expression is not associated with a worse prognosis in the CMS1 group, but is highly prognostic in CMS4 patients for both relapse free and overall survival. Similarly, lack of CDX2 expression was a bad prognostic factor in MSS patients, but not in MSI.

Conclusions: Our work suggests that combination of the consensual CMS classification and lack of CDX2 expression could be a useful marker to identify CMS4/CDX2-negative patients with a very poor prognosis.

Key words: colon cancer, CDX2, consensus molecular subtype classification (CMS)

Introduction

Caudal-type homeobox transcription factor 2 (CDX2) is an important regulator of intestinal development and a biomarker of mature colon epithelial tissues. CDX2 is also involved in colon cancer (CC) oncogenesis [1, 2] and CC without CDX2 expression are often associated with an increased likelihood of aggressive features such as advanced stage, poor differentiation, vascular invasion, BRAF mutation, and the CpG island methylator phenotype [3–6] (see [7] for review).

Recently, Dalerba et al. showed that lack of CDX2 expression was associated with a worse outcome in stage II and III CC patients and that patients with high-risk stage II CC lacking CDX2 appeared to benefit from adjuvant chemotherapy as compared with their CDX2 positive counterpart [8].

Recently again, a consensus molecular subtype (CMS) classification of CC has been reached by merging the data of 6 major publications assessing genomic profiles of thousands of CC patients [9–15]. This consensual classification led to the emergence of four major groups of CC named CMS 1, 2, 3 and 4 with specific molecular profiles and clinical outcomes.

We have analyzed CDX2 mRNA expression in a large multicenter cohort of 469 patients with stage II and III CC according to the threshold proposed by Dalerba et al. (6.5 log2 of normalized expression values) in regard of their CMS classification (GSE39582 series [12]).

Patients and methods

We analyzed whole transcriptome arrays determined on Affymetrix U133 Plus 2.0 chips from a large multicenter cohort of 469 patients with stage II and III CC. All samples were fresh-frozen primary tumor from patients who underwent surgery between 1987 and 2007 in seven centers as previously reported [12]. The GSE33113 dataset (fresh-frozen samples; Affymetrix HG- U133Plus2.0; n = 90) was used as a validation cohort. Data are available via the NCBI Gene Expression Omnibus (http://www.
Results

We found that CDX2 negative CC (i.e. expression \( \leq 6.5 \)) represented 15.6% (73/469) of our study population, which was more frequent than in the work of Dalerba but very close of the percentage found in their validation data set from the NCI-CDP cohort. CDX2 negative samples were significantly enriched in defective DNA mismatch repair tumors \((P = 1.28e-07)\), proximal tumors \((P = 4.14e-06)\), BRAF mutated tumors \((P = 3.06e-07)\) and CMS 1 and 4 groups (supplementary Table S1, available at Annals of Oncology online). Among the 469 tumors, we determined CMS classification for 430 CC samples. Interestingly, 64 of the 68 (94%) CDX2 negative patients belonged to the CMS1 and CMS4 groups \((40/81 \text{ and } 24/101 \text{ patients, respectively; } P < 2.2e-16)\). These two groups are related to microsatellite instability (MSI) and immune infiltration (CMS1) and epithelial-mesenchymal transition (EMT) phenotype (CMS4). The proportion of CDX2 negative CC in the other groups was of 0.5% (CMS2, 1/195) and 5.6% (CMS3, 3/53) (Figure 1). We confirmed these results in an independent cohort (GSE33113) including 90 stage II CC. CMS classification was determined for 79 CC samples, and CDX2 negative CC (i.e. expression \( \leq 6.5 \)) represented 10.1% \((8/79)\) of GSE33113 study population. All the CDX2 negative patients belonged to the CMS1 and CMS4 groups \((7/20 \text{ and } 1/15 \text{ patients, respectively; } P = 0.0003)\) (supplementary Figure S1, available at Annals of Oncology online). In order to validate the prognostic value of CDX2 expression in CMS4 group, we raised the cutoff to 7, which allowed to increase the number of CDX2 negative samples \((CMS1: 9/20, CMS2: 0/31, CMS3: 0/13, CMS4: 4/15; P = 7.686e-05)\) (supplementary Figure S1, available at Annals of Oncology online).

Globally CDX2 expression was an independent prognostic factor \([OS: HR 2.02; 95\% CI (1.27–3.23); P = 0.003; RFS: HR 1.73; 95\% CI (1.06–2.82); P = 0.027]\) in multivariate analysis adjusted for chemotherapy, TNM stage, gender, age, MSI status and tumor location, as previously reported \([8]\) (Table 1). CDX2 expression remains an independent prognostic factor for OS in multivariable model including CMS groups, TNM stage, gender, age, chemotherapy and tumor location (supplementary Table S2, available at Annals of Oncology online).

We thus examined the prognostic impact of CDX2 separately in CMS1 and 4 groups and found that CDX2 status was not prognostic in CMS1 \([OS: HR 0.896; 95\% CI (0.39–2.08); P = 0.798; RFS: HR 0.628; 95\% CI (0.22–1.77); P = 0.378]\) (Figure 2A and B) but highly prognostic in CMS4 patients for both relapse free and OS \([HR: 2.534; 95\% CI (1.29–4.99); P = 0.007; RFS: HR: 2.583; 95\% CI (1.35–4.96); P = 0.004]\) (Figure 2C and D). Similar results were obtained in the validation cohort GSE33113 without reaching statistical significance, due to the small number of events in this series of stage II CC (supplementary Figure S2, available at Annals of Oncology online).

MSI phenotype was overrepresented in CDX2 negative group, due to the enrichment of CMS1 tumors \((P = 1.089e–07)\). However, when we examined specifically in the CMS groups, we showed no correlation between loss of CDX2 expression and MSI status in CMS1 or CMS4 groups (supplementary Table S3, available at Annals of Oncology online).

We examined the prognostic impact of CDX2 expression in MSS versus MSI population. CDX2 status was prognostic in MSS patients for both overall and RFS \((OS: HR 1.966; 95\% CI (1.17–3.31); P = 0.011; RFS: HR 1.72; 95\% CI (1.01–2.93); P = 0.045)\) but not prognostic in MSI \((OS: HR 1.44; 95\% CI (0.55–3.74); P = 0.455; RFS: HR 0.65; 95\% CI (0.17–2.52);\)
P = 0.533) (supplementary Figure S3, available at Annals of Oncology online). Therefore, for samples without CMS status available, combination of CDX2-negative expression and MSS status can be an indication of poor outcome.

**Discussion**

CC is a highly heterogeneous disease. The recent CC molecular classification based on gene expression profiling allowed identifying specific altered biological pathways into the four CMS. Here we identified a loss of CDX2 expression in 2 CMS subgroups: CMS1, characterized by MSI, immune infiltration and good prognosis; and CMS4, showing a mesenchymal/stem cell phenotype and a poor prognosis. Both these groups have been associated with a sessile serrated adenomas (SSA) phenotype–like, as well as an activation of the TGFβ pathway, particularly strong in CMS4 [10–12,16].

In our study we showed that, despite the fact that CDX2 was an independent prognostic factor in the global series, loss of CDX2 expression is not associated with a worst prognosis in the MSI-related CMS1 group. However, in the stem cell-related CMS4 group, CDX2 marker allowed to identify a group of patients with a particularly poor prognosis. In this group lack of CDX2 expression was a poor prognostic factor regardless of BRAF mutational status. On the contrary, prognosis of CMS4/CDX2-positive patients appears essentially identical to that of CMS1 patients.

TGFβ plays a dual role in human cancers. While in normal cells and early carcinomas it acts as a tumor suppressor, in aggressive and invasive tumors it promotes EMT and tumor metastatic spread; activation of the TGFβ pathway has also been linked to resistance to conventional and targeted anti-cancer agents [17, 18]. In a recent work, Fessler et al. showed that TGFβ stimulation in a SSA model strongly reduced CDX2 expression, downregulated expression of genes associated with the classical, epithelial type of CC and induced the expression of mesenchymal marker genes [16]. Therefore, according to the level of the TGFβ signaling activity, sessile serrated adenomas may progress to either the good-prognosis CMS1 or the poor-prognosis CMS4. In poorly differentiated tumors there thus may be two distinct scenarii. In low-differentiated, aggressive CMS4...
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tumors, loss of CDX2 could be a marker of tumor aggressiveness and metastatic potential through high TGFβ production. In low-differentiated, less aggressive CMS1 loss of CDX2 expression may be due to poorly differentiated tumor cells but with a low activation of the TGFβ pathway.

Tumors in the CMS1 sub-group largely coincide with tumors characterized by MSI. Previous studies have observed a statistically significant association between lack of CDX2 expression and reduced survival within the MSI sub-group [19]. Moreover, colon carcinomas characterized by MSI are usually associated with higher survival rates and better prognosis [20]. Large transcriptional multicenter studies as well as cohorts of patients with CDX2 expression levels measured by immunohistochemistry will be necessary to further validate these results. Lately, a CMS classifier based on five immunohistochemical markers has been developed [21], which will likely facilitate clinical translation and stratification in clinical trials testing novel therapies in early-stage CRC.

In conclusion, combination of the consensual CMS classification and lack of CDX2 expression could be a useful marker to identify high-risk patients with poor prognosis. CDX2 does not seem to bear the same prognostic information in the different CMS subgroups, and CDX2 expression status and CMS subgroups represent synergistic biomarkers, most likely to be used in combination. Patients with stage II or stage III CMS4/CDX2-negative colon cancer might be treated differently than others due to their very poor outcome and CMS and CDX2 may be an important stratification factor for future adjuvant trials.

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References


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