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The clinical perspectives of CDX2 expression in colorectal cancer: A qualitative systematic review

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Abbreviations:

APC:	Adenomatous polyposis of the colon
CDX2:	*Caudal*-related homeobox 2
CIMP-H/L:	CpG island methylator phenotype High/Low
CIN:	Chromosomal instability
CLDN1:	Claudin-1
CLDN18:	Claudin-18
EMT:	Epithelia to mesenchymal transition
FAP:	Familial adenomatous polyposis
HEPH:	Hephastin gene
HIF-1α:	Hypoxia inducible factor-1-alpha
HNF4α:	Hepatocyte nuclear factor 4-alpha
IBD:	Inflammatory bowel disease
IHC:	Immunohistochemistry
IRS2:	Insulin receptor substrate 2
LI-cadherin:	Liver-intestine cadherin
LOH:	Loss of heterozygosity
MMR:	Mismatch repair
MSI-H/L:	Microsatellite instable High/Low
MSS:	Microsatellite stable
mTOR:	Mechanistic target of rapamycin
PRISMA:	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RELMβ:	Resistin-like molecule beta
SMARCB1/INI1:	SWI/SNF chromatin remodeling complex subfamily B member 1
SNP:	Single nucleotide polymorphism
TNF-α:	Tumor necrosis factor-alpha
TNM:	Tumor-Node-Metastasis
Wnt:	Wingless-related integration site

Abstract

Homeobox genes are often deregulated in cancer. They can have both oncogenic and tumor-suppressing potential. The *Caudal*-related homeobox transcription factor 2 (CDX2) is an intestine-specific transcription factor, which is restricted to the small intestine and colon in humans after birth. It is implicated in differentiation, proliferation, cell-adhesion, and migration. CDX2 has been proposed as a tumor suppressor in colorectal cancer but its role is still controversial. In order to clarify CDX2s role in colorectal cancer we undertook a literature search in the MEDLINE database from 1966 to February 2014. Only studies on human colorectal cancer tissue were included, thus studies solely performed in cell-lines or animal models were excluded. Fifty-two articles of relevance were identified. We found that CDX2 expression was rarely lost in colorectal cancers, however the expression pattern may often be heterogeneous within the tumor and can be selectively down regulated at the invasive front and in tumor buddings. Loss of CDX2 expression is probably correlated to tumor grade, stage, right-sided tumor location, MMR-deficiency, CIMP, and BRAF mutations. The *CDX2* gene is rarely mutated but the locus harboring the gene is often amplified and may suggest *CDX2* as a linage-survival oncogene. CDX2 might be implicated in cell proliferation and migration through cross-talk with the Wnt-signaling pathway, tumor-stroma proteins, and inflammatory cytokines. In conclusion a clear role for CDX2 expression in colorectal cancer remains to be elucidated, and it might differ in relation to the underlying molecular pathways leading to the cancer formation.

Introduction

Colorectal cancer is one of the most common malignancies in the western world and the second most common cause of cancer related death[1]. The development of cancer is a progressive transformation of normal cells into its malignant counterpart. This involves many known critical mutations in oncogenes and tumor-suppressor genes such as *APC, p53, KRAS*, and DNA mismatch repair (MMR) genes[2–4]. This enables the tumor cells to evade apoptosis, have limitless self-renewal potential and self-sufficiency in growth signaling, as well as invading and metastasizing capabilities into adjacent tissue and organs[3]. In colorectal cancer disruption of the Wnt-signaling pathway often plays a major role in this transformation. In normal colon epithelium Wnt-signaling is involved in preserving tissue homeostasis, but when the signaling pathway is disrupted it leads to accumulation of nuclear β-catenin and increased proliferation[5].

Different molecular pathways, leading to colorectal cancer, have been characterized. An often used classification of phenotypes is between: 1) the chromosomal instability (CIN) pathway and 2) the serrated pathway[6]. The CIN pathway is characterized by: Gain, loss and/or rearrangements of whole chromosomal regions, harboring genes vital to tumor initiation and progression[7]. Features of the serrated pathway is: predilection of right sided colon (proximal to the splenic flexure), $BRAF^{V600E}$ mutations, CpG island methylator phenotype (CIMP) and sometimes, although not required, mutations or loss of expression of MMR proteins[6]. Loss of MMR protein expression leads to accumulation of mutations in microsatellites. On the basis of this the tumors can be characterized as microsatellite stable (MSS) or microsatellite instable high/low (MSI-H/MSI-L)[8,9]. MSI-H tumors does also have predilection for right-sided colon. It is estimated that around 70% originates from the CIN pathway while 30% originates from the serrated pathway[9].

Colon cancer is potential curable by surgical resection of the tumor-bearing segment. The risk of recurrence is reduced in colon cancer stage II and III if treated with adjuvant chemotherapy, but overall survival is not improved for the stage II cancers[10–12]. Thus, there is an ongoing search for new prognostic and predictive biomarkers to stratify these patients into high and low risk groups to determine who might have a survival benefit from adjuvant chemotherapy[13,14].

Caudal-related homeobox 2 (CDX2) is an intestine-specific transcription factor essential for intestinal development and differentiation[15]. Human *CDX2* transcription is

restricted to the adult small intestine and colon[16,17]. It has been proposed to govern expression of numerous genes implicated in various processes such as proliferation, cell adhesion, migration, and tumorigenesis[18]. Homeobox genes are often deregulated in cancer, however they might have both oncogenic and tumor-suppressing potential[19]. This complicates the mapping of their exact roles in the cancer development and progression. Based on mice colon cancer models, CDX2 has been proposed as a tumor suppressor in colorectal cancer[20,21], but its role is still controversial.

This systematic review was undertaken to review the literature with regard to CDX2 expression in colorectal cancer tissue and its possible implications in cancer development, proliferation, and migration. Only studies where the authors performed or or verified their results in human colorectal cancer tissue were included.

Methods

The present analysis was based on the principles of qualitative systematic reviews as described by PRISMA[22,23]. Search strategy, population of interest, and in- and exclusion criteria as described below. Since only retrospective studies and case series have been reported on the subject, a meta-analysis was precluded.

Search strategy

The literature was searched in the MEDLINE database from 1966 up to February 2014 using the following MeSH terms (and free text terms on the combination): "cdx 2"[All Fields] OR "cdx2"[All Fields] AND "colon cancer"[All Fields] OR "colorectal cancer"[All Fields] OR "tumor suppressor"[All Fields] OR "oncogene"[All Fields]. Cross-reference to the referenced articles were made.

Study in- and exclusion criteria

Only English-language articles were evaluated and only original articles were included, thus excluding reviews. Case reports were also excluded. Only studies on human colorectal cancer tissue were included, thus studies performed solely in cell lines or animal models were excluded. Studies, investigating CDX2 expression in other cancer forms than colorectal cancer or in normal tissue without relation to colorectal cancer, were excluded. As were studies dealing with diagnosing of carcinoma of unknown origin. Studies only focusing on CDX1 expression were also excluded.

Results

From a pool of 260 potential relevant articles, 52 were included in this review according to the inclusion criteria (Fig. 1). The studies were subdivided into expression studies, genetic mutation studies, and studies combining laboratory experiments on mice or cell lines with human tissue staining/measurements. Results are summarized in Table 1, 2, and 3. A few studies represented data relevant in more than one category; however, these are only represented once in the tables and figures.

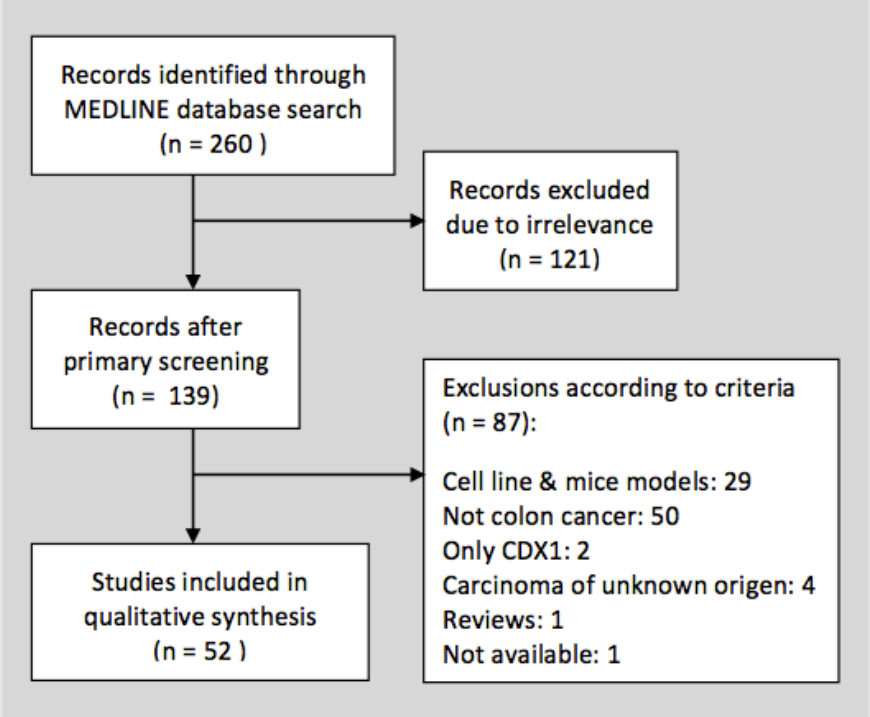

Figure 1: Flowchart of study selection: n: number of studies

Expression studies

Twenty-nine expression studies were included, see Table 1.

CDX2 expression is probably never lost in adenomatous polyps and only rarely lost in colorectal adenocarcinomas, showing strong nuclear staining in up to 90% of cases on immunohistochemical (IHC) staining[24–27]. This makes it a very sensitive marker of adenocarcinomas of intestinal origin[26]. While CDX2 in normal colonic mucosa is restricted to nuclear staining, in cancer tissue the staining often extends into the cytoplasm, Fig. 2C-D[28].

Also the staining can be diffuse or focal Fig. 2A-B[29]. When comparing the tumor center with the invasive front, CDX2 protein expression was significantly lower at the invasive front[30].

When investigating *CDX2* mRNA levels from tumors compared to healthy adjacent tissue a few studies have reported *CDX2* to be significantly decreased in tumor tissue[31,32], while others have reported *CDX2* mRNA to be up-regulated in most tumors[33]. Moreover it was found that CDX2 protein levels and gene regulatory activity were elevated in tumors with elevated *CDX2* mRNA[33].

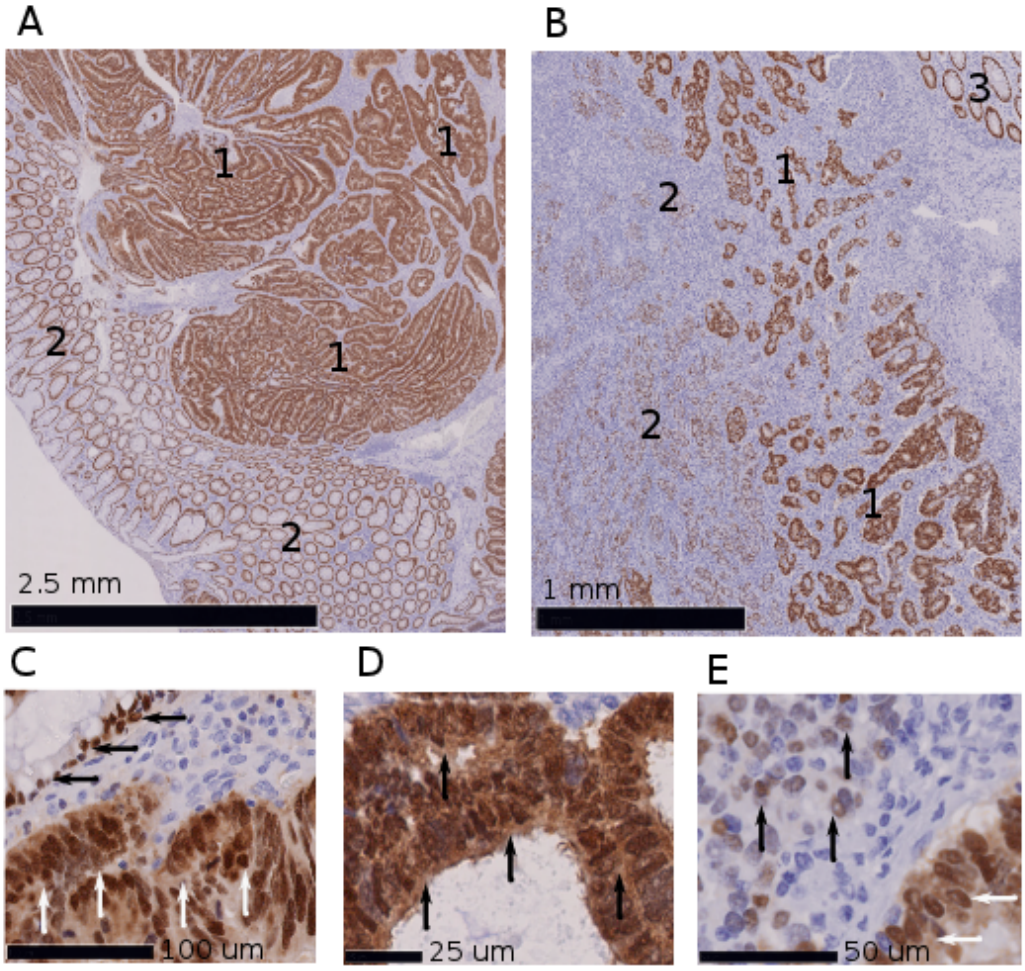

Figure 2
Examples of immunohistochemistry staining, showing various patterns of CDX2 expression in colon cancer tissue. Size bar shown on each picture indicating scale in millimeters (mm) and micrometers (um). **A:** Diffuse and homogenous staining of CDX2 expression in a colon cancer tumor; 1) Cancer tissue 2) Normal tissue. **B:** Focal and heterogonous staining of CDX2 expression in a colon cancer tumor; 1) Cancer tissue with dark staining, 2) cancer tissue with light staining, 3) Normal tissue. **C:** Cancer tissue with nuclear and cytoplasmic CDX2 staining (white arrows) compared with nuclear staining in normal epithelial tissue (black arrows). **D:** Dark cytoplasmic and nuclear staining of CDX2 in colon cancer cells (black arrows). **E:** Heterogeneous staining of CDX2, with light nuclear staining in some cells (black arrows) and darker nuclear and cytoplasmic staining in others (white arrows).

Relation to tumor grade and stage

Several studies have found an inverse correlation between dysplasia grade and CDX2 protein expression, ranging from 75-100% positive staining at low grade dysplasia (high differentiation) to 20-80% at high grade dysplasia (low differentiation)[26,34–39]. The same inverse relationship has been shown for CDX2 expression and cancer stage, classified according to Dukes or TNM[26,35,36,40]. Loss of expression was, in a few studies, a prognostic marker for poor outcome[29,37,41] but not when adjusted for tumor stage and differentiation[37]. Two studies have found that especially medullary colorectal carcinomas (a subgroup of poorly differentiated carcinomas) are prone to loss of CDX2 expression[38,42]. Loss of CDX2 expression may also be correlated with a subset of poorly differentiated colorectal tumors termed "large cell minimally differentiated carcinomas"[34].

Right versus left sided tumors

Loss of CDX2 protein expression has been linked to right sided tumors in several studies[37,43–46]. In two studies, loss of CDX2 expression in right sided tumors was with higher risk of relapse[37,47], while this was not the case in other studies[34,44]. CDX2 expression is lost in about 15% of MSI-H colon cancers[48]. Though loss of expression in most studies seem to be independent of MSI status[37,43,45,49], loss of CDX2 expression in MSI-H patients was in several studies associated with poor prognosis[37,43,48]. Other studies have, however, found a strong correlation between MMR-deficient tumors and loss of CDX2 expression[29,46,50,51]. When evaluating patient relapse and survival some studies find no correlation to CDX2 loss[46,50], while one study found that there was a correlation to unfavorable outcome in MMR-deficient tumors[29], again providing contradictory results.

Loss of CDX2 protein expression has been shown in several studies to correlate with CIMP-H status and in one study inversely correlated to global hypomethylation[37,46,48,49,51]. Loss of CDX2 expression was also correlated to $BRAF^{V600E}$ mutations[44,46,48,51] especially in right sided tumors while it might not be the case in the left sided tumors[44].

Table 1. Expression studies

Ref	Author	Year	Pts (n)	Technique	Comparison	Main findings
[27]	Ee et al.	1995	9	WTS IHC	Norm adj. tissue	CDX2 nuclear staining lost in cell with high degree nuclei atypia.
[32]	Mallo et al	1997	12	Northern blot mRNA	Norm adj. tissue	CDX2 expression down regulated in CRC.
[34]	Hinoi et al.	2001	40	WTS IHC	Norm adj. tissue	Loss of CDX2 expression in 73.3% of LCMDCs
[24]	Moskaluk et al.	2003	60	TMA IHC	None described	CDX2 nuclear staining in 90% of CRC.
[25]	Werling et al.	2003	75	4mm TS IHC	Norm adj. tissue	High CDX2 expression in 99% of CRC.
[26]	Kaimaktchiev et al	2004	1109	TMA IHC	Unrelated tissue	CDX2 expression in 86% of CRC. inversely correlated with stage & grade.
[45]	Okon et al.	2004	58	WTS IHC	Norm adj. tissue	Loss of CDX2 expression correlated with right-sided tumor but not MSI status.
[31]	Kim et al.	2005	48	qPCR mRNA	Norm adj. tissue	CDX2 expression down regulated in CRC.
[33]	Witek et al.	2005	30	qPCR mRNA	Norm adj. tissue	CDX2 expression up regulated in CRC.
[35]	Choi et al.	2006	123	TMA IHC	Norm adj. tissue	CDX2 expression in 76% of CRC. inversely correlated with stage and grade.
[36]	Bakaris et al.	2008	44	4mm TS IHC	Norm adj. tissue	CDX2 expression in 88% of CRC. inversely correlated with stage and grade.
[50]	Lugli et al.	2008	1420	TMA IHC	Unrelated tissue	Loss of CDX2 expression correlated with MMR-deficiency, but not outcome.
[37]	Baba et al.	2009	598	TMA IHC	Unrelated tissue	CDX2 expression in 71% of CRC. inversely correlated with stage, grade, CIMP-H.
[40]	Zheng et al.	2009	80	WTS IHC	Norm adj. tissue	CDX2 expression in 80% of CC. inversely correlated with stage and grade.
[42]	Winn et al.	2009	49	TMA IHC	Norm adj. tissue	Loss of CDX2 expression in 45 and 81% of PDC and MC respectively.
[38]	Winn et al.	2010	69	TMA IHC	Norm adj. tissue	Loss of CDX2 expression in 25 and 60% of PDC and MC respectively.

Ref	Author	Year	Pts (n)	Technique	Comparison	Main findings
[43]	Minoo et al.	2010	399	TMA IHC	Unrelated tissue	Loss of CDX2 expression correlated with right-sided tumor but not MSI status.
[41]	Matsuda et al.	2010	569	TMA IHC	Norm adj. tissue	CDX2 expression in 80% CRC. inversely correlated to survival and Claudin-18 expression.
[44]	Zlobec et al.	2010	600	TMA IHC	Unrelated tissue	Loss of CDX2 expression correlated with BRAF-mutations in right-sided tumors
[30]	Karamitopoulou et al.	2011	220	TMA IHC	Unrelated tissue	CDX2 is down-regulated at the invasive front compared to the tumor center
[49]	Zlobec et al.	2011	337	TMA IHC	Unrelated tissue	Loss of CDX2 expression correlated with CIMP, but not MSI status.
[52]	Laurent et al.	2011	72	WTS IHC	None described	CDX2 expression inversely correlated with β -catenin expression
[47]	Bauer et al.	2012	247	Microarray data set	None described	CDX2 expression inversely correlated to poor outcome in right-sided tumors.
[39]	Knösel et al.	2012	402	TMA IHC	Norm adj. tissue	CDX2 expression inversely correlated with grade and venous invasion
[46]	Dawson et al.	2013	220	TMA IHC	None described	CDX2 expression inversely correlated to MMR-def., CIMP-H and BRAF-mutations.
[29]	Dawson et al.	2013	201	WTS IHC	None described	Loss of CDX2 expression correlated with MMR-def. and poor outcome.
[48]	Kim et al.	2013	109 MSI-H	TMA IHC	None described	Loss of CDX2/CK20 expression correlated with poor outcome
[51]	Walsh	2013	120	WTS	Unrelated tissue	Loss of CDX2 expression correlated with MMR-def., CIMP and BRAF mutations.
[53]	Pancione	2013	134	TMA IHC	Unrelated tissue	Low CDX2 expression is associated with SMARCB1/INI1 negative tumors

Studies evaluating CDX2 expression in colorectal cancer. Pts(n): number of patients/tumors in the study. TMA: Tissue micro array. TS: Tissue section. WTS: whole tissue section. IHC: Immunohistochemistry. qPCR: Qualitative polymerase chain reaction. Norm adj.: Normal adjacent. CC: colon cancer. CRC: colorectal cancer. LCMDC: Large minimally differentiated carcinoma. PDC: Poorly differentiated carcinoma. MC: Medullary carcinoma. CIMP-H: CpG island methylater phenotype high. MMR-def: Miss match repair deficiency. MSI: Micro-satellite instability.

Correlations between CDX2 expression and other colonic expressed proteins

In addition to the above expression studies, linking CDX2 to various morphological and molecular subtypes, CDX2 protein expression has also been reported to correlate with several other genes expressed in colon epithelial cells.

One study reported that CDX2 expression was inversely correlated to cytoplasmic β-catenin at the invasive front in sporadic (but not inflammatory bowel disease (IBD)-associated) tumors[52]. CDX2 expression was also inversely correlated to claudin-18 (CLDN18) expression – a component of tight junctions and proposed oncogene[41]. High CLDN18 expression was a negative predictor of survival[41]. Additionally, CDX2 expression positively correlated with resistin-like molecule β (RELMβ) – an intestinal goblet specific protein – and RELMβ was a positive predictor of survival[40]. Moreover, CDX2 was inversely correlated with several mucins, i.e. MUC2, MUC5AC, and MUC6[51]. Loss of CDX2 expression was furthermore associated with decreased expression of SMARCB1/INI1 – a subunit of the chromatin remodeling complex – which might lead to change in transcription of several genes[53].

Genetic studies

Twelve genetic studies were included, see Table 2.

Germ line mutations

Several studies have investigated single-nucleotide polymorphism (SNPs) within the *CDX2*-gene. Two early studies showed 3 and 6 independent variants of the *CDX2* gene, of which two were considered to be possible disease-associated[54,55]. Later studies have compared SNPs in colorectal cancer patients to healthy individuals. They found no difference in frequency of colorectal cancer between *CDX2* heterozygotes and homozygotes[56–60]. Furthermore, no observed haplotype were associated with increased risk of colorectal cancer, although some were associated with more advanced disease[61]. Polymorphisms in possible splice sites were also investigated and no alterations in mRNA splicing were observed[57]. The incidence of loss of heterozygosity (LOH) of the *CDX2* gene in tumors was in two of these studies, only approximately 10%.

The results from *Cdx2* heterozygote mice, developing hamartoma-like polyps, lead to an investigation of mutations in the *CDX2*-gene in juvenile polyposis and Peutz-Jeghers syndrome patients. Again no pathogenic germ line mutations were found [62].

Somatic mutations

Two studies investigated somatic mutations in colorectal tumors. None of these found any evidence of functional significant SNPs[28,63]. One study reports a frame shift mutation within the *CDX2* gene of a MSI-H patient[64]. The patient had developed antibodies against CDX2 but tumor cells still had positive nuclear IHC staining.

Table 2. Genetic studies

Ref	Author	Year	Pts (n)	Main findings
[55]	Wicking et al.	1998	85 tumor and norm adj tissue	Two SNPs identified in coding region of cdx2.
[56]	Yagi et al.	1999	78 tumor 48 healthy	Somatic mutations and LOH is a rare event in sporadic and HNPCC CC.
[54]	Lin et al.	2000	50 healthy	Three SNPs identified in coding region of cdx2.
[57]	Sivagnanasundaram et al.	2001	51 tumor 60 healthy	Six SNPs identified in the cdx2 gene, none were deemed disease associated. LOH is a rare event.
[62]	Woodford-Richens et al.	2001	47 cases 51 healthy	Mutations in the cdx2-gene do not play a role in juvenile polyposis and Peutz-Jeghers.
[63]	Hinoi et al.	2003	45 tumor	Four missense sequence alterations, none deemed of functional significance
[64]	Ishikawa et al.	2003	27 patients	One frameshift mutation in MSI-H patient who developed antibodies against CDX2
[58]	Rozek et al.	2005	35 tumor 455 healthy	Nine SNPs identified, one in a coding region of cdx2, none were deemed disease associated.
[60]	Slattery et al.	2007	2365 tumor 2969 healthy	CDX2 polymorphism within the VDR CDX2 gene was not deemed disease associated.
[28]	Subtil et al.	2007	35 tumor	Chromosome 13q is often amplified in sporadic CC of CIN phenotype.
[59]	Xia et al.	2009	126 tumor 126 healthy	Three SNPs identified in coding region of cdx2. None were deemed disease associated.
[65]	Salari et al.	2012	226 tumors	Chromosome 13q12 is often amplified in CC in a lineage specific manner.

Studies evaluating genetic mutations and alterations. Pts(n): number of patients/tumors and healthy controls in the study. Norm adj.: Normal adjacent. CC: colon cancer. SNP: Single nucleotide polymorphism. LOH: Loss of heterozygosity. HNPCC: Hereditary non-polyposis colon cancer. CIN: Chromosomal instability. MSI-H: Micro-satellite instability high.

Amplifications and rearrangements

When colorectal tumors were investigated for chromosomal imbalances one of the most common alterations found were gain of chromosome 13q12, harboring the *CDX2* gene, seen in 50% of cases of colorectal cancers investigated[65]. Further investigations showed that this amplification were only significant in colorectal derived tumors, emphasizing its linage specific function. Another study found a similar amplification of the *CDX2* locus but the amplification was not correlated to CDX2 expression when evaluated by IHC[28].

Combined studies

Eleven studies combining laboratory experiments on mice or cell lines with human colon cancer tissue measurements were included, see Table 3.

CDX2 in Wnt/β-catenin signaling and proliferation

Several studies have proposed a connection between CDX2 expression and Wnt-signaling. By IHC staining of colon cancer tissue it has been shown that CDX2, APC, and β-catenin expression were correlated[52,66]. Especially at the invasive front, loss of CDX2 expression seems to inversely correlate with nuclear β-catenin[66]. Additional investigations on colon cancer cell lines proposed that CDX2 binds to the promoter region of APC and AXIN2, activating the transcription, hereby leading to increased degradation of β-catenin[66].

CDX2 might also be implicated in cell cycle control at the G1-S transition, in a non-transcriptional manner. This effect may be mediated by stabilizing $p27^{kip1}$ (a cell cycle inhibitor protein) from ubiquitylation mediated degradation, thus inhibiting cell division[67]. Cancer cell line studies and IHC staining of colon cancer tissue further demonstrated a positive correlation between CDX2 and $p27^{kip1}$ expression levels.

CDX2 also seems to be implicated in the regulation of insulin receptor substrate-2 (IRS2) – a less well characterized component of the insulin growth factor signaling pathway – in a partly APC-dependent manner[68]. In adenomas and cancer specimens from Familial Adenomatous Polyposis (FAP) patients, mRNA levels of *IRS2* and *CDX2* were reduced and correlated to each other[68]. This might switch the cells from absorption and metabolism back to proliferation.

CDX2 in cell adhesion and migration

An implication for CDX2 in migration and metastasis has been proposed based on IHC staining analysis of colon and rectum tumor specimens[66,69]. It was shown that CDX2 was selectively down regulated at the invasive front and in tumor buddings compared to the central tumor areas.

Cadherins are implicated in cell-cell adhesion and regulation[70,71]. Loss of expression might lead to disruption of cell-cell contact, promoting migration and epithelia to mesenchymal transition (EMT)[72]. CDX2 and liver-intestine cadherin (LI-cadherin) expression have been shown to strongly positively correlate by IHC of both normal and colon cancer tissue[73]. Further cell line and knock-out mice experiments suggests that this regulation is a result of direct binding of CDX2 to the *LI-cadherin* promoter[73].

Contradicting CDX2 as a tumor suppressor in modulating cell-cell contact is a study showing positive correlation with CLDN1 – a constituent of tight junctions that is associated with a poor outcome, in colorectal cancer tissue [74]. Further cancer cell line experiments showed that the expression was also partly controlled by functional cross talk with the Wnt-signaling pathway[74].

CDX2 and the tumor environment

Since significant mutations in the *CDX2* gene seems rare, it has been proposed that the tumor environment may play a role in the altered levels of expression of CDX2. A study engrafting human colon cancers freshly surgically resected, into the subcutis and the cecal wall of nude-mice, showed differential expression pattern with regard to the site of implantation[75]. Thus, the tumors showed heterogeneous expression pattern at the time of surgical resection but when subcutaneous injected into the mice, an increased homogeneous expression was observed. When injected in the cecal wall, the growth and expression pattern of CDX2 resembled that of the original tumor[75].

Changes in CDX2 expression might also be influenced by collagen type I, normally expressed in stroma, and restricted from contact with epithelial cells by the basement membrane[69]. Tumor cells, which were disconnected from the basement membrane and thus in contact with collagen type I, showed loss of CDX2 expression by IHC staining. Furthermore, collagen type I induced a 55% reduction in *CDX2* mRNA in a colon cancer cell line experiment[69].

Tumor necrosis factor-alpha (TNF-α), an inflammatory cytokine, was also inversely correlated with CDX2 expression at the invasive front and in tumor buddings in rectal cancer specimens. Furthermore, treatment with TNF-α was able to reduce CDX2 expression in a dose dependent manner to 50% in colon cancer cell lines[76].

Oxygen supply might also induce altered expression patterns of CDX2. Hypoxia-inducible factor-1α (HIF-1α) overexpression is associated with tumor hypoxia and might be implicated in angiogenesis and migration[77]. It was shown by IHC that CDX2 was inversely correlated with the expression of HIF-1α. A decreased *CDX2* mRNA expression was further seen in colon cancer cell lines when hypoxia was induced[77].

Table 3. Combined studies

Ref	Author	Year	Pts (n)	Model	Main findings
[73]	Hinoi et al.	2002	40	Mice and cell line: HT-29, HEK293	LI-cadherin is regulated by CDX2.
[69]	Brabletz et al.	2004	45	Mice and cell line: Caco-2, DLD1, LS174T	Collagen type I induces down-regulation of CDX2.
[80]	Hinoi et al.	2005	148	Cell line: HT29, WiDr, RKO, LS147T, MCF-7	HEPH and intracellular iron levels are regulated by CDX2
[75]	Benahmed et al.	2007	24	Mice and cell line: HT-29, SW480, T84	CDX2 expression various with sight of implantation.
[68]	Modica et al.	2009	-	Mice and cell line: Caco-2, HT29, SW480	IRS2 is regulated by CDX2.
[67]	Aoki et al.	2011	59	Mice and cell line: DLD1, LS174T	CDX2 stabilizes $p27^{kip1}$ from degradation.
[77]	Zheng et al.	2010	62	Cell line: SW480, LS174T	HIF-1a induces down-regulation of CDX2.
[74]	Bhat et al.	2012	50	Cell line: HCT116, SW480	Claudin-1 is regulated by CDX2.
[79]	Saandi et al.	2012	35	Mice	CDX2 is regulated by HNF4a
[66]	Olsen et al.	2013	22	Cell line: Caco-2, SW480, RKO	CDX2 regulates APC & AXIN2.
[76]	Coskun et al.	2014	55	Cell line: Caco-2	TNF-α induces down-regulation of CDX2

Studies investigating CDX2 through laboratory models and clinical tumor samples. Pts(n): number of patients/tumors in the study. –: number not stated.

CDX2 expression in relation to other colonic expressed genes

The transcription factor hepatocyte nuclear factor 4-alpha (HNF4α) is involved in intestinal cell homeostasis, architecture, and barrier function[78]. In colon cancer, a correlation between the expression of CDX2 and HNF4α was shown by IHC staining, showing low expression of both, especially in some stretches of invading cells at the tumor edge. This correlation was further confirmed by siRNA knock-down of *HNF4* α in a cell line experiment, which lead to a decrease in CDX2 expression[79].

From expression array data on colon cancer tissue, CDX2 expression was shown to strongly correlate with the *hephaestin*-gene (*HEPH*), which is implicated in iron transport within the intestine[80]. These findings were further investigated in colon cancer cell lines and mice models. It was shown that *CDX2* directly regulates *HEPH* expression in response to intercellular iron levels, leading to increased iron export from the cells[80].

Discussion

CDX2 expression is rarely lost in colon cancer. IHC staining show that CDX2 expression is not solely restricted to the nucleus but often extends into the cytoplasm. Furthermore, the expression pattern can be heterogeneous within the tumor and might be selectively down regulated at the invasive front and in tumor buddings. Loss of CDX2 expression is probably correlated with grade, stage, right-sided location, MMR-deficiency, CIMP, and BRAF mutations. Different haplotypes of the *CDX2* gene is probably not disease related and somatic mutations in the tumor tissue are rare events. However the *CDX2* locus on chromosome 13q12, is often amplified in colon cancers and may suggest *CDX2* as a linage-survival oncogene. CDX2 seems to be implicated in both cell proliferation and migration in colon cancer, in part through a cross-talk with the Wnt-signaling pathway. The contact with tumor stromal might also induce down-regulation of CDX2 and hereby change the expression of cell adhesive proteins and migratory capabilities.

Increasing evidence supports that right-sided and left-sided tumors might differ with regard to predilection of their molecular origin, especially with regard to MMR/MSI-status, CIMP-status, and BRAF mutations[5]. It could therefore be the case that CDX2 might exert different roles in colorectal cancer depending on which molecular pathway was initially involved in the malignant transformation. Loss of CDX2 expression in cancers of the serrated pathway could be an "innocent bystander" or an active part of tumor progression. Supporting that CDX2 down-regulation might be of importance in tumor progression, is evidence of a poorer outcome in those tumors with loss of CDX2 expression[37,43,48]. For chromosomal instability tumors, however, it might be the case, that they are partly reliant on CDX2 expression and that the gene in some cases is amplified and up-regulated[28,81,82]. This is supported by cell line experiments showing that in several colon cancer cell lines, it is impossible to knock-down *CDX2* completely and keep the cells viable[81,83,84].

It is, however, important to bear in mind that many of the IHC studies are performed using tissue microarrays. Here the site from where in the tumor, the core was punched out, was not systematically specified in almost all of the studies. Since CDX2 expression may be focal or heterogonous within the tumor, the results from IHC might be biased. Furthermore, a classification of right versus left or chromosomal instability versus serrated pathway might be to simplistic. Recent studies show significant differences in

survival rates with regard to the precise tumor location in the colon and with regard to underlying molecular phenotypes[85,86].

There is increasing evidence of a cross-talk between CDX2 and the Wnt-signaling pathway[76,84,87–90]. Besides cell line studies indicating that CDX2 directly binds to the *AXIN2* and *APC* promoter region, CDX2 might also through direct or indirect protein interaction with β-catenin lead to disruption of the β-catenin/TCF complex[91,92]. It is possible that CDX2 through up-regulation of the protein Mucdhl, promote localizing of β-catenin to the membrane, making β-catenin less available for translocation to the nucleus[92]. However, the interaction between the Wnt-signaling pathway and CDX2 is probably dynamic, changing with differentiation and in some cases CDX2 might even promote Wnt-signaling[81,83,84]. Besides regulating Wnt-signaling, CDX2 may also reduce proliferation through interaction with cell-cycle proteins, growth factors, and mTOR activity[20,67,93–95]. The role of CDX2 is probably also ambiguous in this matter, and may differ according to conditions. Two cancer cell line studies indicate that CDX2 under anchorage-independent growth conditions (resembling spreading cells) promotes proliferation and increases resistance to programmed cell death[96,97].

A hallmark of invasive cancers is the ability of the cells to escape the tumor and spread to distant organs[98]. In order to do this, morphological changes occur in which the cells down-regulate cell adhesive proteins and dedifferentiate to a stage resembling those in embryogenesis and wound healing, a transformation called EMT[72]. Since CDX2 is involved in the differentiation of epithelial cells along the crypt axis, a down-regulation might lead to de-differentiation of tumor cells[99]. In wound healing assays it has been shown that CDX2 was down-regulated in migrating cells and that forced expression lead to decreased motility and invasive potential[100,101]. Besides regulating LI-cadherin, several cell line studies have shown that CDX2 might promote E-cadherin cell adhesion[102–104]. Furthermore, the tumor milieu might play a prominent role in down-regulation of CDX2. In addition to collagen type-I being able to down-regulate CDX2, several cell line studies suggest that inflammatory cytokines such as TNF-α and cyclooxygenase-2 may also induce down-regulation of CDX2[76,105–108]. In this way CDX2 expression might differ at the invasive front compared to central in the tumor.

CDX2 was originally proposed as a tumor suppressor based on mice studies showing heterozygote *Cdx2*$^{+/-}$ mice developed multiple polyps in the proximal colon[109]. This was first interpreted as precancerous lesions with dysplasia but was later shown to be hamartomas without malignant potential[110,111]. In another mice model, *Cdx2*$^{+/-}$ mice receiving

azoxethane, a DNA mutagen, developed typical adenocarcinomas with invasive properties[112]. In human colon cancer however, the exact role of CDX2 appears more complicated and since mutations in the *CDX2* gene are rare[62,113], the role of CDX2 might be more dynamic and even differ in various colon cancers. Adding to the dynamic role of CDX2 is, that in other gastrointestinal tumors (gastric and esophageal) and leukemia, CDX2 is often characterized as an oncogene[114–116]. More insight into the molecular pathways and the interaction between tumor cells and the tumor milieu is vital to understanding the role of CDX2 in colorectal cancer, and its possible oncogenic and tumor suppressing properties.

In conclusion CDX2 expression is seldom lost in colorectal cancer, but loss of expression is probably correlated to right-sided tumors of the serrated pathway. Down-regulation of CDX2 may happen through interaction with the tumor stroma especially at the invasive front. Through cross-talk with the Wnt-signaling pathway and down-regulation of cell adhesive proteins this might have an affect on proliferation and cell migration. However, a clear role for CDX2 expression in colorectal cancer remains to be elucidated, and at the time it is not suited as a biomarker of prognostic or predictive relevance.

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