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Role of CDX2 Marker in Patients with Colorectal Cancer

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ABSTRACT

CDX2 has been proposed as a tumor suppressor in colon cancer, *CDX2* gene is often amplified in colon cancer, there is a lineage survival oncogene function in some tumors, the role of CDX2 protein during CRC development remains debatable. The aim of this study investigate the effect of low *CDX2* expression on overall survival (OS) for prognosis of CRC patients and estimate of therapeutic activity of CDX2 expression in predictive of chemotherapy respond. This study done in the Middle Euphrates Unit for Cancer Research, Faculty of Medicine, University of Kufa in Al-Najaf province. This study was carried out on sixty-three cases of CRC in the form of available paraffin blocks who underwent surgical resection between 2015 and 2020. Thirty_ seven blocks of normal non tumoral colorectal tissue collected randomly from archives of two private laboratories during collection of malignant tissue blocks. Nuclear low expression of CDX2 in control and patient groups were 0(0.00%) and 30 (47.6%), while high expression of this protein was 37 (100%) and 33(52.4%) in control and patient groups respectively. OS was longer in patients with low CDX2 protein expression who intake adjuvant chemotherapy (71.33%) with mean survival (24.75±2.81 month) than patients were not intake adjuvant chemotherapy (53.71%) with mean survival (15.227±1.66 month). CDX2 expression regarded as diagnostic marker for non-mucinous CRC. High of CDX2 expression can be used as an independent good prognostic biomarker to predict longer survival of patients with CRC.

1. Introduction

Colorectal cancer (CRC) is a major cause of death throughout the world. It is the third most common cancer worldwide after lung and breast cancers and the fifth most common cause of death with approximately 1,148,515 new cases diagnosed and 576,858 related deaths in 2020 (Sung, et al., 2021). In Iraq, it is the eighth commonest cancer representing 8.4% of male cancers and 4.6% of female cancers. Although the diagnosis of CRC is straightforward in the primary site, yet it may represent a diagnostic problem in metastatic tumor of unknown primary origin (Bayrak, Haltas, & Yenidunya, 2012). Therefore, the establishment of reliable diagnostic markers, which confirm or rule out colorectal origin, is mandatory especially in adenocarcinomas of unknown primary site which is one of the most confusing clinical problems (Su, et al., 2008).

Classical prognostic parameters such as histopathological type, histologic grade, and stage are often used to predict the prognosis of CRC, but patients with the same stage or histological grade often exhibit inhomogeneous biological behavior. Moreover, reliable prognostic biomarkers for CRC are not yet available (Xiong, et al., 2018; Slik, et al., 2019). Adjuvant chemotherapy can further reduce the risk of relapse or death in CRC patients but most of the survival benefit is limited to patients with stage III tumors. Thus, scientific community efforts are constantly aimed at identifying new prognostic and predictive biomarkers to stratify patients into high- and low-risk groups and maximize the benefit from adjuvant chemotherapy (Böckelman, et al., 2015).

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Caudal-related homeobox transcription factor 2 (CDX2) is an intestine-specific transcription factor essential for intestinal development and differentiation (Verzi, et al., 2010) and is associated with cell proliferation, migration, and tumorigenesis (Coskun, Troelsen & Nielsen, 2011). It is encoded by CDX2 gene which is a member of the caudal-related homeobox gene family that maps to the ParaHox gene cluster (Gao, White, & Kaestner, 2009) in chromosome 13q12 (Olsen, et al., 2014). The expression of CDX2 in adults is restricted to the intestine, from the duodenum to the rectum, particularly limited to the nuclei of intestinal epithelial cells (Slik, et al., 2019). Although strong nuclear immunoreactivity of CDX2 is seen in the majority of CRC cases, decrease or complete loss in CDX2 expression has been reported in 10-30% of cases (Bae, et al., 2015). In addition, the decrease in CDX2 expression is associated with classical prognostic markers such as histological grading, and stage (Slattery, et al., 2007). CDX2 has been proposed as a tumor suppressor in colon cancer, but CDX2 expression is seldom lost in colon cancer tissue, and the gene is rarely mutated (Slattery, et al., 2007). Furthermore, several studies have found that CDX2 gene is often amplified in colon cancer, suggesting a lineage survival oncogene function in some tumors (Salari, et al., 2012).

The role of CDX2 protein during CRC development remains debatable, as different studies suggest both high (Ryan, et al., 2018) and low expression in CRC patients (Asgari-Karchekani, et al., 2020; Sjoerd, et al., 2021).

2. Materials and Methods

2.1. Study Design

This study was carried out on sixty-three cases of CRC in the form of available paraffin blocks who underwent surgical resection between 2015 and 2020, these data collected retrospectively from the archives of three private laboratories. Two pathologist histologically re-evaluated each pathological material. these data used to estimate CDX2 expression by IHC with mucinous and non-mucinous CRC adenocarcinoma and normal colorectal tissue subjects as control and investigate the correlation between CDX2 expression with there available clinicopathologiecal feature.

2.1. Control group

Thirty-seven blocks of normal non-tumoral colorectal tissue collected randomly from archives of two private laboratories during collection of malignant tissue blocks and re-evaluated by two pathologists to ensure from its normality

2.3. Statistical Analyses

Data have been analyzed by using SPSS version 21 and Pad Graphprism version 7, Mean ± SE were used to represent numerical variables, whilst number and percent were used to express nominal variables. Where data were regularly distributed, Student t-test used for compare the averages of two groups when variables were not normally distributed, the Mann Whitney U test was used to compare the mean of two groups. To compare frequency distributions, the chi-square test was employed, and where it was invalid, the corrected chi-square test was used instead, Risk assessment was performed using an odd ratio with a 95% confidence interval and a etiologic fraction. The Kappa statistic using for calculate concordance rate. The relationships between overall survival (OS) and CDX2 expression were evaluated using the Kaplan-Meier method (log-rank test). Significant results were defined as those with a P-value less than or equal to 0.05.

2.4. Intake Chemotherapy and Survival data

After getting the CRC paraffin blocks from private laboratories, follow up the data of death and chemotherapy intake for these blocks of CRC patients. From AL-Najaf middle Euphrates center of oncology. For chemotherapy intake, 13 patient's intake chemotherapy ,14 no chemotherapy and 36 with no data. On the other hand, we revealed 20 patient's death (event),7 patient's survival (censored) and 36 patients missing. These data used for concluded the role of four years' overall survival (OS) prognosis analysis after diagnosis which measured from the data of surgery until the data of death any cause, patients who were alive at the data of last follow up were censored (Asgari-Karchekani, et al., 2020).

3. Results and Discussion

Immunohistochemical nuclear expression of CDX2 protein in patients with CRC and control group

Nuclear low expression of CDX2 in control and patient groups were 0(0.00%) and 30 (47.6%), while high expression of this protein were 37 (100%) and 33(52.4%) in control and patient groups respectively, so the high expression decreased significantly in patients when compared with control (P=0.001), furthermore, Odd ratio was 2.12; 95% CI was (1.65-2.71).

Table 1
CDX2 nuclear expression in a group of patients with colorectal cancer and a control group

Nuclear CDX2 Expression	control NO (%)	patients NO (%)
Low expression	0 (0.00%)	30 (47.6%)
High expression	37 (100.00%)	33 (52.4%)
Total	37 (100.00%)	63 (100.00%)

Odd ratio (95%CI†) 2.12(1.65-2.71)
P=0.001
CI†= confidence interval

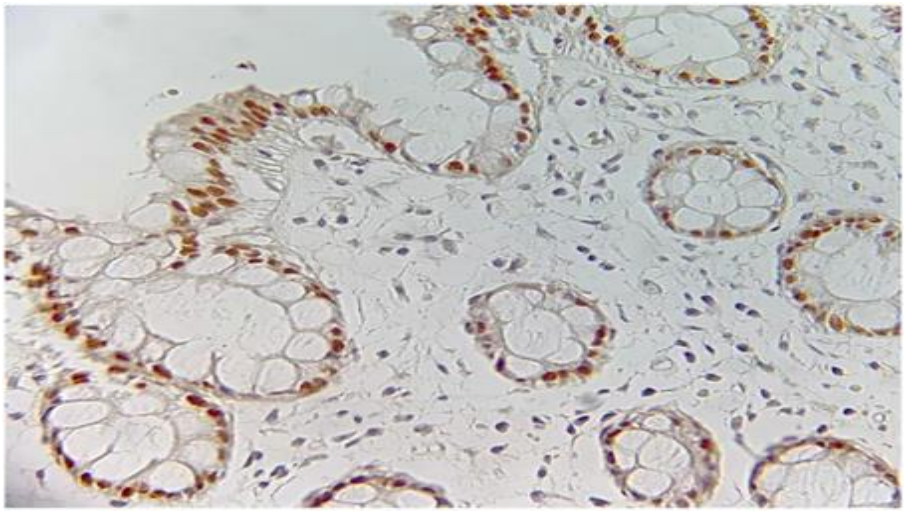

Fig. 1. Normal epithelium show strong positive CDX2

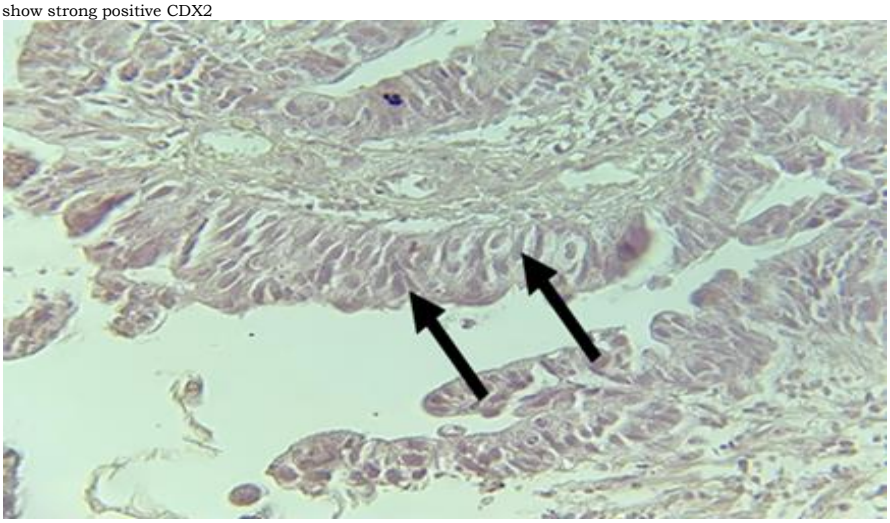

Fig. 2. Adenocarcinoma show negative CDX2

3.1. Association between IHC CDX2 Protein Expression and Clinicopathological Features

3.1.1. Association between Age of CRC Patients and CDX2 Protein Expression

Regarding to age of CRC patients, CDX2 low immunoexpression was detected in 25 patients (58.1%) out of 43 patients (100%) with >50 year while CDX2 low immunoexpression was found in 5 patients (25%) out of 20 patients (100%) with ≤50 year, this result clarified a significant difference (P= 0.014) in low expression of CDX2 between patients with >50 year and ≤50 year as shown in table (2).

3.2. OS in Low and High CDX2 Protein Expression

In Kaplan-Meier analysis, OS was 61.77% in patients with low expression of CDX2 and 79.12% in patients with high expression of CDX2, also, patients with low CDX2 expression showed shorter OS (17.943±1.7 month) than those with high CDX2 expression (33.431±2.7 month) (log-rank P = 0.0001)

Table 2

Overall survival (OS) in low and high CDX2 protein expression

CDX2 protein expression percentage	Mean OS survival ± SE Month	death N (%)	OS Survival (%)	Odd (95%CI†)
Low expression	17.943±1.7	12 (60%)	(61.77%)	
High expression	33.431±2.7	8(40%)	(79 .12%)	2.18 (1.29-3.70)

P=0.0001

CI†=confidence interval

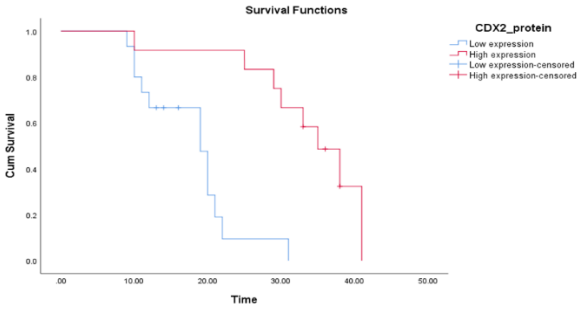

Fig. 3. Kaplan-Meier survival diagram according CDX2 expression status in patients with CRC. OS (P=0.0001) (A); Blue line: low expression. Red line: high expression

3.3. Discussion

CDX2 expression and activity change as colon cells progress along the pathway to cancer (Beck, 2002). The CDX2 is a transcription regulator for a number of genes responsible for cell proliferation, differentiation, and migration (Guo, Suh & Lynch, 2004). It is clinically useful to detect the CDX2 expression level in the tumoral tissue, since it is known to be an almost specific marker for gastrointestinal neoplasms, especially CRC (Barbareschi, et al., 2003). This study revealed that the high expression of CDX2 protein decreased significantly in tissues of CRC patients than control with normal tissue that has no one with low expression of CDX2 while approximately half of CRC patients enrolled in this study has low expression of CDX2. In accordance with the present results, previous studies have demonstrated that the CDX2 expression level in CRC patients was decreased when compared with normal mucosa control (Hinoi, et al., 2001; Matsuda, et al., 2010). In addition to play an important role in the development and differentiation of the intestine, CDX2 has also been known to exert a tumor-suppressor role in CRCs. The tumor-suppressor function of CDX2 in CRCs has been evidenced by an increased susceptibility for tumors in heterozygous Cdx2+/- mice, accelerated G1-S cell cycle transition, and increased chromosomal instability in colon cancer cell lines with reduced levels of CDX2 (Bonhomme, et al., 2003).

On the other hand, this finding is contrary to previous studies which have suggested that the CDX2 protein is overexpressed by CRC compared with adjacent normal (Dae Hong, et al., 2013). overexpression of CDX2 still unknown. Where CDX2 expressed in tumor by the process of neoplastic transformation. Overexpression may reverse the genetic instability characterizing carcinogenesis. Thus, 35% colorectal tumors appear on chromosome 13q12.13-q12.2 where the CDX2 gene encoding (Douglas, et al., 2004).

Furthermore, most colorectal cancers express nuclear factor-nB constitutively, a potent transactivator of CDX2 expression (Kojima, et al., 2004). overexpression of CDX2 may change mechanisms of equilibrium responsible for differentiation, proliferation, and survival properties of epithelial cells that is disrupted during carcinogenesis. hyperplastic colonic polyps in CDx2+/_ mice result from inactivation of CDX2 in epithelial cells results in the formation of (Bonhomme, et al., 2003). Conditional expression of CDX2 in colon cancer cells, on the other hand, inhibited their growth in vitro (Mallo, et al., 1998).

During colorectal carcinogenesis the mechanisms for loss of CDX2 expression are still unclear. CDX2 mutations placed infrequently in CRC with defective DNA mismatch repair (i.e., MSI-high). In a population-based case-control study, CDX2 polymorphisms do not play a role in reduced CDX2 expression (Sullivan, et al., 2008). Although not a major cause, loss of heterozygosity at the CDX2 gene (13q12-13) may explain for CDX2 expression loss in a small proportion of CRC patients (Sivagnanasundaram, et al., 2001). An epigenetic change, such as promoter CpG island methylation, may be the cause of CDX2 silence, according to a study employing colon cancer cell lines that found evidence for a dominant transcriptional repressor of CDX2 (Hinoi, Loda, & Fearon, 2003).

4. Conclusions

CDX2 expression regarded as diagnostic marker for non-mucinous CRC, also High of CDX2 expression can be used as an independent good prognostic biomarker to predict longer survival of patients with CRCs.

Competing Interests

The authors have declared that no competing interests exist.

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