Immunohistochemical staining of cytokeratin 20 and cytokeratin 7 in colorectal carcinomas: Four different immunostaining profiles

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INTRODUCTION

Worldwide, colorectal carcinoma (CRC) is associated with significant mortality and morbidity. In Saudi Arabia, CRC accounted for 11.9% of all newly diagnosed patients in 2013. CRC ranked first among men and the third most common cancer among women. Recently, molecular studies demonstrated that CRC is a heterogeneous group of diseases that develop through three main

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pathogenetic pathways; the chromosomal instability pathway (constituting 60–70% of sporadic CRC), the microsatellite instability (MSI) pathway (accounts for about 15% of sporadic CRC), and the CpG island methylation pathway. Tumours originating through these three pathways differ in precursor lesions, natural history, and pathological features. The immunohistochemical characteristics of molecular subsets of CRC are not well studied. [3–6]

The CK20+/CK7− profile is characteristic for colonic carcinoma and successfully used to distinguish it from tumours originating from breast, gynaecological tract, liver or lung. However, not all CRC expressed the usual cytokeratin profile, as some studies reported strong CK7 expression and conversely negative CK20 immunoeexpression. CK20+/CK7− profile is expressed in about 75–95% of CRC, while the rest of cases show different profiles. [7–11] Previous studies correlated cytokeratin expression to clinicopathological characteristics of CRC. They found that loss of CK20 is associated with older age (above 56), right colonic tumour, higher grade, increased intratumoral lymphocytic infiltration (creating Crohn’s disease-like infiltrate), mucinous histology, advanced tumour stage, presence of lymph node metastasis and worse overall and disease-free survival compared with patients with positive CK20 expression. [6,12,13]

The objective of this study was to explore patterns of CK20/CK7 immunostaining in primary CRC and nodal metastasis in a set of Saudi Arabian patients, and to analyse the diagnostic, prognostic and predictive role of variable patterns of CK20/CK7 immunostaining.

**MATERIALS AND METHODS**

**Patients**

One hundred and forty-four retrospective CRCs and 49 corresponding regional nodal metastasis constituted the material of the present study. Pathological materials were collected from archives of Pathology Department, King Abdulaziz University Hospital, Jeddah, Saudi Arabia in the period 1995–2012. The diagnosis of CRC was confirmed after excluding the possibility of carcinomas of non-colonic origin by re-evaluation of clinical presentation, endoscopic and radiological findings as well as serum tumour markers (CEA, CA19-9, CA125 and AFP) and immunohistochemistry for Cdx 2, vimentin, Ca-125 whenever needed. Clinical data was retrieved from the patients’ records. Clinicopathological parameters of patients are summarised in Table 1. Postoperative pathological staging was performed according to the AJCC staging system. [14] The study was approved by the Research Committee of the Biomedical Ethics Unit, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. All patients included in this study gave an informed written consent for utilisation of their material in research.

| Parameter                              | Number (%) |
|----------------------------------------|------------|
| **Sex**                                |            |
| Male                                   | 72 (50%)   |
| Female                                 | 72 (50%)   |
| **Grade**                              |            |
| Well-differentiated                    | 35 (24.3%) |
| Moderately-differentiated              | 89 (61.8%) |
| Poorly-differentiated                  | 20 (13.9%) |
| **Age**                                |            |
| <60 years                              | 78 (54.2%) |
| ≥60 years                              | 66 (45.8%) |
| **Tumour location**                    |            |
| Right colon                            | 39 (27.1%) |
| Left colon                             | 90 (62.5%) |
| Rectum                                 | 15 (10.4%) |
| **Tumour size**                        |            |
| <5 cm                                  | 59 (41%)   |
| ≥5 cm                                  | 85 (59%)   |
| **Primary tumour**                     |            |
| T1                                     | 4 (2.8%)   |
| T2                                     | 20 (13.9%) |
| T3                                     | 108 (75%)  |
| T4                                     | 12 (8.3%)  |
| **Nodal metastasis**                   |            |
| Positive                               | 61 (42.4%) |
| Negative                               | 78 (54.1%) |
| Cannot be assessed                     | 5 (3.5%)   |
| **Distant metastasis**                 |            |
| Positive                               | 42 (29.2%) |
| Negative                               | 102 (70.8%)|
| **Lymphovascular invasion**            |            |
| Positive                               | 26 (18%)   |
| Negative                               | 118 (82%)  |
| **Surgical resection margins**         |            |
| Involved                               | 12 (8.3%)  |
| Free                                   | 132 (91.7%)|
| **Survival**                           |            |
| Died of disease                        | 33 (22.9%) |
| Alive                                  | 96 (66.7%) |
| Not available                          | 15 (10.4%) |
| **Local disease recurrence**           |            |
| Recurrence                             | 38 (26.4%) |
| No recurrence                          | 106 (73.6%)|

**Table 1: Clinicopathological parameters of patients (n=144)**

T1: Tumour invades submucosa; T2: Tumour invades muscularis propria; T3: Tumour invades through the muscularis propria into the subserosa or into nonperitonealized pericolic or perirectal tissues; T4: Tumour directly invades other organs or structures and/or perforates visceral peritoneum.

**Tissue microarray construction**

Tissue microarray (TMA) was constructed from CRC and nodal metastasis paraffin blocks using an automated tissue arrayer (MASTER 3D HISTECH). Two tissue cores (each 1.5 mm) were punched from two different target areas of each donor block. TMA blocks then sliced into 4-μm thick sections and mounted on salinated slides for further immunohistochemistry.
Immunohistochemistry of tissue microarray
Immunoperoxidase technique was performed using monoclonal mouse Anti-Human CK-20 and monoclonal mouse Anti-Human CK-7 (Dako Cytomation Norden A/S, Glostrup, Denmark, dilution 1:100 each). An automatic immunostainer (Ventana Bench Mark XT, Ventana Inc., Tucson, AZ) was used following the manufacturer’s instructions. In each analysis, positive controls consisted of CRC samples previously shown to stain with CK20 and thyroid tissue known to be positive to CK7. Tris-buffered saline in place of the primary antibody was used as a negative control.

Interpretation of immunohistochemical staining
Cells were considered positive for CK20 and CK7 when distinct cytoplasmic and/or cell membrane yellow to brown staining was identified. Percentage of positive cells were recorded in a semiquantitative method according to a scale from 1 to 4; score 4 (staining in >50% of tumour cells), score 3 (staining in 20–50% of cells), score 2 (staining of 5–20% of cells), while score 1 (<5% staining). When CK20 immunostaining was dichotomized for statistical risk assessment, scores 1 and 2 were defined as low immunostaining, while scores 3 and 4 were included in high immunostaining category. In case of CK7, score 1 was considered as low immunostaining, while scores 2, 3 and 4 were considered as high immunostaining.

In addition, the combination of immunostaining of CK20/CK7 was considered into four classes as follows: CK20+/CK7−, CK20−/CK7−, CK20+/CK7+, and CK20−/CK7+. Negative was defined as low immunostaining and positive was defined as high immunostaining.

Statistical analysis
Mann–Whitney test was used to test the difference between two variables. The Kruskal–Wallis test was used to demonstrate the association between three or more groups of patients. Non-parametric Chi-square was used to test variance along one variable. Logistic regression analysis was used to predict nodal metastasis, distant metastasis, surgical resection margins, and lymphovascular invasion. Disease-free survival probabilities were tested by Kaplan–Meier procedure. Disease-free survival time was calculated from the date of pathological diagnosis to the occurrence of relapse. Statistical procedures were performed using SPSS® version 16.0 (SPSS, Chicago, IL, USA). Statistical significance was determined at P value of ≤0.05 and was two-sided.

RESULTS

CK20 and CK7 immunostaining profile
Positive cytoplasmic and membranous immunostaining of CK20 was seen in 62.5% of primary CRC and 63.5% of regional nodal metastasis [Figure 1 and Table 2]. CK7 immunostaining was seen in 5.6% of primary CRC and in 4% of regional nodal metastasis [Figure 1 and Table 2]. In primary CRC, CK20 immunostaining was statistically significantly higher than CK7 immunostaining (P < 0.001). On the other hand, there was no statistically significant difference in CK20/CK7 immunostaining noted between CRC and lymph node metastasis. The combination of CK20/CK7 immunoprofile showed that the CK20+/CK7− profile was the highest followed by CK20−/CK7− then CK20+/CK7+ and CK20−/CK7+ (2.1%). Details of results are shown in Table 3.

Relation between CK20/CK7 immunostaining and prognosis
There was no association between CK20/or CK7 immunostaining and clinicopathological features. Regression analysis revealed no predictive or prognostic value of CK20 and/or CK7 immunostaining in CRC [Table 4]. CK20 immunostaining was not related to disease free survival, Log rank (Mantel–cox) = 1.435, P value = 0.231 as
was CK7 immunostaining; Log rank (Mantel-cox) = 0.000, $P$ value = 0.996 [Figure 2].

**DISCUSSION**

Relative expression of CK20/CK7 is an approved diagnostic tool to help determine site of origin in metastatic carcinomas. CK20 is specific for colonic, urothelial and Merckel cell carcinoma. On the other hand, CK7 is characteristic of glandular malignancies originating from breast, respiratory tract, biliary tract and Mullarian epithelium. CK7 expression in CRC is rare and positivity considered as exclusion criteria to tumours of CRC origin.[7,8,11] Occasionally, loss of expression of CK20 and conversely positive expression of CK 7 was noted in some CRC. The value of this aberrant expression is still unclear.[15–17] We studied the immunostaining patterns of CK20/CK7 in 144 CRC and in 49 lymph node metastasis. In primary CRC, CK20 was positive in 62.5%, while CK7 was positive in 5.6%. In nodal metastasis, CK 20 and CK 7 showed positivity in 63.5% and 4.1%, respectively.

In the current study, we classified CRC tumours into four groups according to different CK20/CK7 immunostaining profiles. The most common profile was the usual CK20+/CK7− (60.4%), followed by negativity to both markers (35.4%), followed by positivity to both markers (2.1%) and the CK20−/CK7+ profile (2.1%) with minor discrepancy. In the present CRC tumours showed increased percentage of CK 20 negativity and reduced percentage CK7 positivity. The reason for this discrepancy could be explained by difference in the studied population, technical variations in immunohistochemical procedure or interpretation criteria. The reason behind unusual immunostaining of CK20 and CK7 is still unclear. Recent molecular studies categorised CRC into microsatellite stable and microsatellite instable tumours. Many studies have attempted to find the relationship between immunophenotypic and molecular backgrounds of CRC.[18–21] Others studied the CK20/CK7 expression in 44 CRC cases in relation to molecular subtypes.[19,20] They concluded that reduction or total absence of CK20 is a phenotypic characteristic of CRC originating through MSI. On the other hand, Gurzu and Jung[12] a conducted similar study and reported that microsatellite instability-CRCs are associated with diffuse expression of CK7 and absence of CK20. The above results can explain the reason behind aberrant expression of CK20 and CK7 in percentage of CRC. We compared percentage of four CK20/CK7 profiles in CRC obtained in the present research to some previous researches in Table 5.

The relative expression of CK20/CK7 in malignant tissue was compared to clinicopathological characteristics. We

### Table 2: Categories of CK20 and CK7 immunostaining

|                  | Primary tumour (n=144) | Nodal metastasis (n=49) | $P$  |
|------------------|------------------------|-------------------------|------|
|                  | CK20                  | CK7                     | CK20 | CK7    |
| Low immunostaining | 54 (37.5%)            | 136 (94.4%)             | 13 (26.5%) | 47 (95.9%) | (CK20=0.165) |
| High immunostaining | 90 (62.5%)            | 6 (4.2%)                | 36 (63.5%) | 2 (4.1%) | (CK7=0.979) |
| $P$ value        | 0.003*                | <0.001*                 | 0.001* | <0.001* |

*One sample nonparametric Chi-square test, $\Rightarrow$ Mann–Whitney test

### Table 3: Differential CK20/CK7 immunostaining in primary CRC patients

| CK20/CK7 profile | n (%) |
|------------------|-------|
| 1 CK20+/CK7−     | 87 (60.4%) |
| 2 CK20+/CK7+     | 3 (2.1%) |
| 3 CK20−/CK7−     | 51 (35.4%) |
| 4 CK20−/CK7+     | 3 (2.1%) |
| Total            | 144 (100%) |

### Table 4: Regression analysis for CK7 and CK20 immunostaining

| Variable                | Exp (B) | 95% CI for exp (B) | $P$  |
|-------------------------|---------|--------------------|------|
| Nodal Metastasis        |         |                    |      |
| CK7                     | 1.288   | 0.176–9.416        | 0.803|
| CK20                    | 0.863   | 0.432–1.723        | 0.677|
| Distant metastasis      |         |                    |      |
| CK7                     | 0.473   | 0.054–4.177        | 0.501|
| CK20                    | 0.727   | 0.345–1.151        | 0.395|
| Local disease recurrence|        |                    |      |
| CK7                     | -0.125  | 0.638–0.088        | 0.136|
| CK20                    | 1.213   | 0.588–2.635        | 0.626|
| Lymphovascular invasion|         |                    |      |
| CK7                     | 0.098   | -0.129–0.506       | 0.243|
| CK20                    | 0.532   | 0.226–1.255        | 0.150|

Figure 2: Disease-free survival (a) According to CK20 immunostaining; Log rank (Mantel-cox) = 1.435, $P$ value = 0.231. (b) According to CK7 staining; Log rank (Mantel-cox) = 0.000, $P$ value = 0.996. Time is calculated in months
found no difference in CK20 and CK7 immunostaining by age, sex, tumour location, size, histological grade, presence of lymphovascular invasion, positive margins, lymph node status or tumour stage or tumour progression indicators. Our results are concordant with those of Hernandez et al, except that their findings showed an association between CK20+/CK7+ profile and advanced CRC in comparison to early stage CRC, which commonly show CK20+/CK7− profile. On the other hand, Park et al claimed that CK20−/CK7+ profile is associated more with right side CRC than left side tumours, and most cases consisted of high-grade carcinoma. Bressenot et al suggested that aberrant expression of CK20 and CK7 is related to tumour progression, based on studying one of his cases. The case was of a 70-year-old lady presenting with high-grade CRC, stage T3, N2, MX. The primary tumour was CK7+/CK20−, whereas, tumour metastasizing to lymph nodes showed variable profiles of CK20+/CK7− and CK20−/CK7−. Moreover, the authors suggested that aberrant CK20/CK7 profile is usually found in CRC metastasizing to lung, ovary and endometrium.

CONCLUSIONS

Our results may help in demonstrating the heterogeneity of CRC. According to CK20/CK7 immunostaining, CRC were categorised into four different subclasses. Unfortunately, we could not correlate between these subclasses and clinicopathological variables, survival outcome or prognostic data. A considerable number of CRC expressed aberrant immunoprofiles of CK20/CK7, which should be considered during diagnosing carcinomas in metastatic regions. Further studies on larger cohorts correlating different immunohistochemical cytokeratin profiles to molecular subtypes of CRC are recommended for better understanding of pathogenesis, and different behaviour of CRC, which dictates the likelihood response to traditional and targeted therapeutic agents.

Clinical practice points

- CRC shows different combinations of CK20/CK7 immunostaining.
- The reason behind unusual immunostaining of CK20 and CK7 is still unclear.
- Aberrant CK20/CK7 immunostaining should be considered when used in diagnosis of metastatic CRC.

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Conflicts of interest

There are no conflicts of interest.

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