Investigation of Insulin-Like Growth Factor-1 (IGF-1), P53, and Wilms’ Tumor 1 (WT1) Expression Levels in the Colon Polyp Subtypes in Colon Cancer

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Background: There is no study in the literature investigating the expression levels of WT1, p53, and IGF-1 in colon polyp subtypes. In this study, we aimed to investigate the expression levels of IGF-1, p53, and WT1 in colon polyp subtypes and to determine whether expression levels are correlated with each other.

Material/Methods: Tissue specimens were obtained from 105 patients (80 men, 25 women; age range, 30–91 years) who underwent surgical resection for colorectal cancer (CRC) at Ordu University School of Medicine, Department of Pathology between January 2015 and 2017. Parameters such as age, sex, region of origin, and pathological diagnosis type were determined. The preparations were immunohistochemically stained with corresponding markers.

Results: The results of the study showed that there was a statistically significant relationship between WT1 expression (negative – positive) in polyps and the place where the sample was taken (P=0.011). There is a positive relationship between P53 staining score (0–3) and positive frequency of IGF-1 (60.9–85.7%). There was a statistically significant change in P53 scores and location (P=0.006, p=0.015, respectively). As the P53 score of the polyps increased (0 to 3), the rate of adenomatous (34.8–78.4%) increased, so a positive relationship was found. WT1 and IGF-1 gene expression was associated with tumor location, p53 staining score, and sex.

Conclusions: WT1 and IGF-1 are appropriate markers for CRC, and WT1 expression in CRC primary tumors especially could be a novel independent marker for prognosis and tumor progression.

MeSH Keywords: Colonic Neoplasms • Genes, p53 • Immunotherapy • Insulin-Like Growth Factor I • Wilms Tumor

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Gastrointestinal polyps are proliferative and neoplastic lesions originating from the mucosa and submucosa epithelium and protruding into the lumen of the gastrointestinal tract. Gastrointestinal (GIS) polyps are more commonly seen in the colorectal region [1]. The polyps detected by the colonoscopic examination may be stemmed or unaltered and their size is variable [2,3]. Colorectal polyps are classified as non-neoplastic polyps (hyperplastic (metaplastic) polyps, hamartomatous polyps (juvenile polyposis, Peutz-Jeghers syndrome, Cronkhite-Canada syndrome, Cowden syndrome), inflammatory polyps), neoplastic polyps, adenomas (tubular, tubulovillous, villous). Adenomas may include mild, moderate, and severe dysplasia. Tubular adenomas are usually small and show mild dysplasia. As the diameter of polyps increases, dysplasia increases in villous patients. We found 88% mild, 8% moderate, and 4% severe dysplasia in tubular adenomas; 58% mild, 26% moderate, and 16% severe dysplasia in tubulovillous adenomas; and 41% mild, 38% moderate, and 21% severe dysplasia in villous adenomas [4,5]. Insulin-like growth factor-1 (IGF-1) shows the in vitro effect either acutely on the anabolic effect on protein and carbohydrate metabolism, or on long-term cell differentiation. IGF-1 stimulates DNA synthesis, and cell replication in cell cycle is a very important effect. It has been shown that cell turnover increases the risk of cellular transformation by the action of IGF-1 receptor [6], insulin-like growth factor-1 (IGF-1) and its receptor (IGF-1R) are also thought to play a key role in mitogenesis and tumorigenesis [7,8]. In addition to prognostic factors, histopathological biomarkers such as insulin-like growth factor (IGF), androgen receptor status, microvascular density, Ki-67 index, and p53 gene mutation are also reported to be useful in predicting disease progression [9–11]. P53 stress signals in a somatic cell are involved in apoptosis, cell cycle stop, DNA repair, and aging, and has an important role in translating into classical processes such as “the protector of the genome” [12,13]. Inactivation of the P53 tumor suppressor gene occurs in more than half of all human tumors, meaning that loss of this gene is an important step in the pathogenesis of cancer [14].

P53 and Wilms’ tumor 1 (WT1) are tumor suppressor genes that are expressed everywhere and mutated in a large proportion of human cancers [14]. Changes in WT1 expression have been described in other malignancies and premalignant syndromes [15,16]. Most solid tumor cell lines were examined; WT1 expression was found to be increased in lung, gastric, and breast cancers [17]. There are also studies showing that WT1-expressing leukemia and solid tumor cells are inhibited by the treatment of WT1 antisense oligomers and that the WT1 gene has an oncogenic function in these malignancies [17,18]. These results indicate that WT1 may be a tumor antigen whose expression could be increased for use in immunotherapy against both leukemia and solid tumors [19]. The ability of the WT1 protein to function as a target antigen for immunotherapy has been studied in vivo and in vitro in human systems [20–22]. The results showed that WT1 protein may be a promising tumor rejection antigen for cancer immunotherapy [22,23].

There is no study in the literature investigating the expression levels of WT1, p53, and IGF-1 in colon polyp subtypes, and their relationship with age, sex, region of origin, and pathological diagnosis.

In this study, we aimed to investigate the expression levels of IGF-1, p53, and WT1 in colon polyp subtypes and to determine whether expression levels are correlated with each other. However, in order to prevent the occurrence of cancer, we need to maximize the efficacy of immunotherapy in patients after polyp resection.

### Material and Methods

#### Patients and surgical specimens

The experimental protocol was approved by the Institutional Human Ethics Committee of Ordu University (approval no: 2017/46). Tissue specimens were obtained from 105 patients (80 men, 25 women; age range, 30–91 years) who underwent surgical resection for colorectal cancer at Ordu University School of Medicine, Department of Pathology between January 2015 and 2017. Parameters such as age, sex, region of origin, and pathological diagnosis type were determined. The preparations were immunohistochemically stained with corresponding markers.

#### Immunohistochemical analysis

Kits used in the histochemical analysis are CellMarque 453M-94 p53 (DO7) Conc. 0.1mL (1: 100–500), CellMarque 348M-94 WT1-Wilms’ Tumor (6F-H2) Conc. 0.1 mL (1: 100–500), and EMD Millipore CBL52 Insulin-like Growth Factor-I (M23) 100 ug. Immunohistochemical analysis of tissue sections was reviewed by 2 pathologists independently, and a consensus had to be reached in cases of interobserver variation. The pathologists were blinded to the clinicopathological features of the specimens. Specimens were considered immunopositive for P53 when 10% or more of tumor cells showed clear evidence of nuclear staining. Weidner criteria for analysis were used [24]. IGF-1, P53, and WT1 protein staining were analyzed semi-quantitatively. Stained cells were counted under a light microscope (magnification, ×400) and the percentage of stained cells was counted in 5 visual fields. The average was then calculated and divided according to the scoring levels.
positively-stained cells, 0; <25% positively-stained cells, 1; 26–50% positively-stained cells, 2; >50% positively-stained cells, 3.

Statistical analysis

All statistical analyses were performed using SPSS Statistics 25 (IBM SPSS, NY, USA). The gene expression levels in cancer tissue were compared with those in adjacent normal mucosa using the Wilcoxon signed-rank test. The associations between gene expression and potential explanatory variables (including age, sex, tumor location, histological type, expression levels, and staining scores) were evaluated using the chi-square test. A p-value of <0.05 was considered significant.

Results

There was no significant difference between the age distribution of the patients in terms of the presence of WT1 expression (P=0.974) (Table 1).

Histochemical staining of p53, IGF-1, and WT1 is shown in Figure 1. Nuclear staining with p53 in HP, membrane staining with IGF-1 in AP, and cytoplasmic/membranous staining with WT1 in HP are shown in Figure 1. The results of the study showed that there was a statistically significant relationship between WT1 expression (negative-positive) in polyps and the place where the sample was taken (P=0.011). WT1-positive staining was strongest in samples taken from the pre-transverse colon section and weakest in samples taken from the post-transverse colon (Table 2). There was no statistically significant relationship between WT1 expression in the polyps and sex, diagnostic IGF, or P53 staining scores, but there were clinically significant differences in OR (odds ratio) values. Positive staining according to hyper-polyposis (HP) was 1.609 times (OR=4.308) higher in the adenomatous polyposis (AP), 1.393 times (OR=1.393) higher in the women compared to men, and those with IGF-positive staining were 2.103 times (OR=2.103) higher than in those with IGF-negative staining. In addition, we found a positive relationship between WT1 expression (0–3) and WT1 positive frequency (39.1–64.3%). In other words, as the WT1 staining score increased, the rate of WT1 staining increased.

There was no statistically significant relationship between IGF-1 expression (negative-positive) and P53 staining scores (P=0.062). The IGF-1 positive staining rate was highest in the samples with the most severe staining (Table 3). However, there was a positive relationship between P53 staining score (0–3) and positive frequency of IGF1 (60.9–85.7%). In other words, the P53 staining score increased as the staining rate increased. Our findings showed that there was no relationship between sex, WT1, and IGF-1 staining in cases where the samples were hyperplastic and adenomatous after pathological diagnosis. We found that there was no relationship between sex, WT1, and IGF-1 staining in patients with hyperplastic and adenomatous lesions after pathological diagnosis. However, there was a statistically significant correlation between P53 scores and location (P=0.006, p=0.015, respectively). As the P53 score of the polyps increased (0–3), the rate of adenomas (34.8–78.6%) increased, so a positive relationship was found. The rate of AP pathological diagnosis was 88.2% in the samples taken from the transverse colon. No statistically significant difference was found between the mean age of the patients diagnosed with AP and HP (P = 0.060). Although there was no significant relationship between pathologic diagnosis and sex, in terms of OR values, the rate of adenomatous pathologic diagnosis was 2.60 times higher than hyperplastic pathologic diagnosis, 2.69 times higher in men compared to women, and 2.075 times higher in patients with IGF-1 staining than in negative ones (Table 4).

The results of the study revealed that there was no significant relationship between P53 status and WT1 (P=0.473) and IGF-1 (P=0.740) staining status in the samples (Table 5).

There was no statistically significant correlation between P53-IGF-1-WT1 status and AP and HP status (P=0.088). However, AP and HP incidence rates were significantly higher in P53-positive patients than in others (Table 6). In the P53-positive group, IGF-1 positivity was higher in patients with IGF-1-positivity than in those with IGF-1-positivity, but negative or positive WT1 status was not associated with AP and HP distribution (Table 6).

Discussion

We found that that the colon location in the colon polyps affected the WT1 positivity and the adenomatous polyp ratio, and that the p53 staining score, WT1, and IGF-1 positivity increased in adenomatous polyps. However, as the p53 score increased, WT1 and IGF-1 positivity increased. In addition, the WT1 positivity rate was 2.371 times higher in females, and in men the adenomatous polyp ratio was 1.609 times and IGF-1 was 2.075 times higher.

The P53 gene is a proven tumor suppressor gene, is downregulated in colon cancer patients, and is consistent with malignant transformation, as reported in the literature. Hundreds of target genes have been associated with p53, including WT1 and IGF-1 [25–30].

The WT1 protein was shown to be expressed in cancer cells derived from various kinds of cancers, including colon cancer, breast cancer, primary leukemia, bone and soft-tissue sarcoma, lung cancer, and head and neck squamous cell
Table 1. Age distribution by WT1 staining status.

| WT1 staining status | n  | Mean   | SD   | P-value |
|---------------------|----|--------|------|---------|
| No                  | 56 | 60.95  | 11.09| 0.974   |
| Yes                 | 48 | 61.02  | 11.88|         |

Figure 1. Histochemical staining of p53, IGF-1, and WT1. Nuclear staining with p53 in HP. Membrane staining with IGF-1 in AP and cytoplasmic/membranous staining with WT1 in HP.
A study by Miyata et al. found that WT1 expression was increased in colorectal cancers. Furthermore, WT1 expression was reported to be correlated with tumor progression, lymph node metastasis, and clinical staging [35]. Oji et al. [32] showed that WT1 expression in colon cancer was significantly higher than that in normal-appearing colorectal tissues, with no significant correlations between WT1 expression and clinical factors and TNM stages. In the present study, WT1 gene expression was found to be associated with tumor location, p53 staining score, and sex.

A number of previous studies have compared the expression levels of the IGF-1, IGF-2, IGF-1R, and IGFBP-3 genes in colon cancer tissue and adjoining normal mucosa. Zhang et al. [36] showed that there was identifiable IGF-1 mRNA in malignant human colonic tissue. The mRNA expression levels of the IGF-1R gene were reported to be higher in adenocarcinoma tissue of the colon compared to adjoining normal mucosa [37]. Another study demonstrated that mRNA expression of the IGF-1R gene was detected in liver metastasis in CRC tissue specimens but was undetectable in adjoining normal mucosa [38]. Keku et al. [5] demonstrated that the mRNA expression level of the IGF-1R gene was higher in CRC tissue compared with adjoining normal mucosa.

### Table 2. Sex, pathological diagnosis, location of sample, and WT1 distribution according to IGF and P53.

| Features                        | WT1 status | χ²-value | P-value |
|--------------------------------|------------|----------|---------|
|                                | Negative n (%) | Positive n (%) |        |
| Sex                            |            |          |         |
| Men                            | 45 (56.3)  | 35 (43.8) | 0.522   | 0.470   |
| Women                          | 12 (48.0)  | 13 (52.0) |          |         |
| Pathological diagnosis         |            |          |         |
| Hyper-polyposis (HP)            | 28 (60.9)  | 18 (39.1) | 1.430   | 0.232   |
| Adenomatous polyposis (AP)      | 29 (49.2)  | 30 (50.8) |          |         |
| Location of sample             |            |          |         |
| Before transverse colon         | 5 (27.8)   | 13 (72.2) |          |         |
| Transverse colon                | 7 (41.2)   | 10 (58.8) | 9.095   | 0.011   |
| After Transverse colon          | 45 (64.3)  | 25 (35.7) |          |         |
| IGF-1                           |            |          |         |
| No                              | 25 (65.8)  | 13 (34.2) | 3.176   | 0.075   |
| Yes                             | 32 (47.8)  | 35 (52.2) |          |         |
| P53 staining score              |            |          |         |
| None (0)                        | 14 (60.9)  | 9 (39.1)  |          |         |
| Mild (1)                        | 22 (55.0)  | 18 (45.0) | 2.448   | 0.485   |
| Moderate (2)                    | 16 (57.1)  | 12 (42.9) |          |         |
| Severe (3)                      | 5 (35.7)   | 9 (64.3)  |          |         |

### Table 3. IGF distribution by P53 staining score.

| PS3 staining score | IGF-1 status | χ²-value | P-value |
|--------------------|--------------|----------|---------|
|                    | Negative n (%) | Positive n (%) |        |
| None (0)           | 9 (39.1)     | 14 (60.9) |          |         |
| Mild (1)           | 12 (30.0)    | 28 (70.0) | 7.332   | 0.062   |
| Moderate (2)       | 15 (53.6)    | 13 (46.4) |          |         |
| Severe (3)         | 2 (14.3)     | 12 (85.7) |          |         |

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mucosa. However, the mRNA expression levels of the IGF-1 gene were reduced in cancer tissue compared with adjoining normal mucosa. Peters et al. [39] showed that IGF-1 gene expression was not associated with any clinicopathological characteristic in CRC, while Shiratsuchi et al. [40] reported that IGF-1 gene expression in CRC was associated with tumor size, depth of tumor invasion, lymphatic invasion, and venous invasion in CRC. Increased postoperative tumor growth and the presence of liver metastasis were associated with significantly elevated IGF-1R gene expression in gastrinoma [40,41]. In the present study, IGF-1 gene expression was associated with tumor location, p53 staining score, and sex.

### Table 4. According to the pathological diagnosis (hyperplastic and adenomatous) WT1, IGF-1, and P53 distribution.

| Features          | Pathological diagnosis | χ²-value | P-value |
|-------------------|------------------------|----------|---------|
|                   | Negative n (%) | Positive n (%) | |
| Sex               |                        |          |         |
| Men               | 31 (38.8) | 49 (61.3) | 3.494 | 0.062 |
| Women             | 15 (60.0) | 10 (40.0) |        |       |
| WT1               |                        |          |         |
| Negative          | 28 (49.1) | 29 (50.9) | 1.430 | 0.232 |
| Positive          | 18 (37.5) | 30 (62.5) |        |       |
| IGF-1             |                        |          |         |
| No                | 21 (55.3) | 17 (44.7) |        |       |
| Yes               | 25 (37.3) | 42 (62.7) | 3.174 | 0.075 |
| P53 staining score|                        |          |         |
| None (0)          | 15 (65.2) | 8 (34.8)  |        |       |
| Mild (1)          | 21 (52.5) | 19 (47.5) | 22.382 | 0.006 |
| Moderate (2)      | 7 (25.0)   | 21 (75.0) |        |       |
| Severe (3)        | 3 (21.4)   | 11 (78.6) |        |       |
| Location of sample|                        |          |         |
| Before transverse colon | 9 (50.0) | 9 (50.0) | 8.461 | 0.015 |
| Transverse colon  | 2 (11.8)   | 15 (88.2) |        |       |
| After transverse colon | 35 (50.0) | 35 (50.0) |        |       |
| Age*              | 58.9 (9.11) | 62.8 (12.7) | −1.90 | 0.060 |

* Age values were given as mean (std deviation) according to pathological diagnosis groups.

### Table 5. Distribution of P53 according to IGF-1 and WT1 status.

| Features  | P53 staining | χ²-value | P-value |
|-----------|--------------|----------|---------|
|           | Negative n (%) | Positive n (%) | |
| WT1 status|                        |          |         |
| Negative  | 14 (24.6) | 43 (75.4) | 0.514 | 0.473 |
| Positive  | 9 (18.8)  | 39 (81.3) |        |       |
| IGF status|                        |          |         |
| Negative  | 9 (23.7)  | 29 (76.3) | 0.110 | 0.740 |
| Positive  | 14 (20.9) | 53 (79.1) |        |       |
We acknowledge that there are some limitations in our study. These include a small sample size, the relatively small proportion of high-grade and advanced-stage lesions, and lack of survival data. Despite these limitations, the WT1 and IGF-1 expressions may reflect tumor progression and metastatic activity. These analyses of WT1 and IGF-1 will increase our understanding of CRC pathogenesis, and could further lead to novel treatment and early diagnostic tools and offer new strategies for immunotherapies in CRC.

Table 6. Comparison of P53-IGF-1-WT1 and AP-HP distribution.

| P53 | IGF-1 | WT1 | AP | HP | \( \chi^2 \)-value | P-value |
|-----|-------|-----|----|----|-------------------|--------|
| –   | –     | 2   | (1.90) | 5   | (4.76) |        |
|     | +     | 0   | (0.00) | 2   | (1.90) |        |
| +   | –     | 3   | (2.86) | 4   | (3.81) |        |
|     | +     | 3   | (2.86) | 4   | (2.81) |        |
| +   | –     | 10  | (9.52) | 8   | (7.62) |        |
|     | +     | 5   | (4.76) | 6   | (5.71) |        |
| +   | –     | 14  | (13.33) | 11  | (10.48) |        |
|     | +     | 22  | (20.95) | 6   | (5.71) |        |

Conclusions

WT1 and IGF-1 are appropriate markers for CRC, and WT1 expression in CRC primary tumors especially could be a novel independent marker for prognosis and tumor progression.

Conflict of interests

None.
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