The Prognostic Significance of Global Histones Deacetylation in Colorectal Cancer Patients Treated with Fluoropyrimidine Therapy

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**ABSTRACT**

To study the impact of fluoropyrimidine (FP) therapy on the acetylation level of histones (H3 and H4), and its correlation to treatment outcome in colorectal cancer (CRC) patients. Total histone protein was extracted, and the level of global histones (H3 and H4) acetylation was determined in the peripheral blood of 66 CRC patients before treatment with FP therapy, and also in 48 and 32 of those patients after 3 and 6 months of treatment, respectively. Clinicopathological stratification of patients was conducted for subgroup analysis. After three years of follow up, event-free survival (EFS) and the hazard of recurrence and progression were determined by Kaplan−Meier and COX regression analyses, respectively. Baseline CRC patients showed global H3 hyperacetylation by 160%, but H4 hypoacetylation by 87% relative to healthy control. FP therapy significantly reduced global H3 and H4 acetylation levels especially in subgroups of CRC patients > 45 years, females, with right colon tumours, with normal baseline levels of CEA and CA19.9, with negative lymph nodes and negative metastasis, and also in the patients who showed no signs of recurrence or progression after three years of follow up. Survival analyses showed decreased median EFS time and increased the hazard of recurrence and progression in patients with high CEA (HR= 3.38, P= 0.023), positive metastasis (HR= 1.16, P<0.001), and H4 hypoacetylation <87% (HR= 1.55, P=0.014). FP therapy-induced reduction in the global level of histones acetylation declared in subgroups of CRC patients with excellent prognostic features.

**INTRODUCTION**

Colorectal cancer (CRC) is the most frequently diagnosed type of cancer in developing populations (Arnold et al., 2017). CRC progresses in a multistep pattern results from the accumulation of genetic and epigenetic aberrations under microenvironmental influences. Epigenetic regulators as DNA methylation, histone modifications, chromatin remodelers and non-coding RNAs, are the mostly known transcriptional regulators which regulate the inflammatory transformation of colonic mucosa into carcinoma (Amirkhah et al., 2019; Yang et al., 2019). The diagnosis, prognosis and treatment of CRC are primarily affected by the post-translational modifi
Table 1: Clinicopathological characters of 66 CRC patients

| Patients Subgroups | N   | %    |
|--------------------|-----|------|
| Age                |     |      |
| <=45               | 31  | 47.0%|
| >45                | 35  | 53.0%|
| Sex                |     |      |
| Female             | 30  | 45.5%|
| Male               | 36  | 54.5%|
| Baseline CEA       |     |      |
| Normal             | 49  | 74.2%|
| High               | 17  | 25.8%|
| Baseline CA19.9    |     |      |
| Normal             | 60  | 90.9%|
| High               | 6   | 9.1% |
| Site of tumor      |     |      |
| Right Colon        | 47  | 71.2%|
| Left colon and rectum | 19 | 28.8%|
| T                  |     |      |
| T2                 | 17  | 25.8%|
| T3                 | 40  | 60.6%|
| T4                 | 9   | 13.6%|
| Negative           | 42  | 63.6%|
| Positive           | 24  | 36.4%|
| M                  |     |      |
| Negative           | 50  | 75.8%|
| Positive           | 16  | 24.2%|
| Response to FP therapy (After 3 years of follow up) | | |
| No event of recurrence and progression | 33 | 50.0% |
| Event of Recurrence & Progression | 33 | 50% |

Data presented as count and percentage (%) of CRC patients stratified in subgroups according to their clinicopathological features. N: number of patients, CEA: Carcinoembryonic antigen, CA19.9: carbohydrate antigen 19.9, T: tumor burden, N: lymph node, M: metastasis.

Acetylations (acetylation, methylation and phosphorylation) of histones nuclear proteins (Qin et al., 2019). The acetylation of histones regulates gene transcription through the fine-tuning between active (euchromatin) and inactive (heterochromatin). Neutralizing the basic charge of lysine residues with acetylation is controlled by two main enzymes: histone acetyl-transferase enzymes (HATs) and histone deacetylase enzymes (HDACs) (Bowman and Poirier, 2015).

Fluoropyrimidine (FP) based therapy is still the standard of treatment in CRC. It is described as a single agent of capecitabine or 5-fluorouracil (5-FU), and in combination with oxaliplatin (Dekker et al., 2019). Many CRC patients may develop resistance to 5-FU during treatment, which is associated with a high rate of recurrence and poor survival (Longley et al., 2003). Epigenetic alterations play a significant role in drug resistance in various cancers, including CRC (Crea et al., 2011), in addition to the changes induced by FP- therapy to the global epigenetic machinery of the patient (Fouad et al., 2018). In our previous study, the overall level of DNA methylation was significantly reduced by FP- based therapy, causing a significant deterioration in the overall and event-free survival of CRC patients (Fouad et al., 2018).
Table 2: Global H3 acetylation in subgroups of CRC patients at baseline and after treatment with FP therapy

| Patients Subgroups | at baseline | Global H3 acetylation % after 3 months of FP therapy | % after 6 months of FP therapy | P-Value |
|--------------------|-------------|-----------------------------------------------------|-------------------------------|---------|
|                    | N Median(IQR) | N Median(IQR) | N Median(IQR) | |
| Age                | ≤45          |            |                |            |         |
|                    | 31           | 165.10(125.06-220.00) | 20 | 123.00(105.23-156.14) | 13 | 112.10(98.37-125.00) | 0.368 |
|                    | >45          | 35         | 146.33(123.39-187.92) | 28 | 120.15(105.55-129.21) | 19 | 117.20(103.90-139.25) | 0.003 |
| Sex                | Female       | 0.192      | 162.70(123.90-214.09) | 0.580 | 121.54(105.23-129.42) | 0.266 | 0.036 |
|                    | Male         | 0.282      | 140.19(123.66-190.47) | 0.839 | 121.05(106.54-140.54) | 0.863 | 0.368 |
| Baseline CEA       | Normal       | 0.15930    | 159.30(123.39-181.40) | 0.11871a | 118.71(97.41-128.00) | 0.10667a | 0.004 |
|                    | High         | 0.17596    | 159.06(125.06-190.06) | 0.12461 | 124.61(109.26-156.88) | 0.11673 | 0.0276 |
| Baseline CA19.9    | Normal       | 0.440      | 141.92(119.89-218.46) | 0.386 | 107.07a(100.00-128.00) | 0.402 | 0.043 |
|                    | High         | 0.0461     | 168.14(123.82-206.04) | 0.38247 | 129.42(119.30-155.00) | 0.194 | 0.368 |
| Site of tumor      | Right Colon  | 0.0968     | 148.84(123.49-190.06) | 0.427 | 121.54a(103.50-128.00) | 0.194 | 0.015 |
|                    | Left colon and Rectum | 0.194 | 178.00(125.06-234.00) | 0.12160 | 117.91(107.60-156.14) | 0.147 | 0.233 |
|                    | P- value     | 0.569      | 148.84(123.49-190.06) | 0.015 | 121.54a(103.50-128.00) | 0.194 | 0.015 |

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| T  | T2  | 17  | 12  | 9   | 0.311 |
|----|-----|-----|-----|-----|-------|
|    |     |     |     |     |       |
|    |     | 159.06 | 121.54 | 106.86 |
|    |     | (123.82-187.92) | (94.15-150.00) | (94.15-184.97) |
| T3 |     | 146.35 | 117.50 | 115.68 |
|    |     | (124.44-195.84) | (105.23-128.00) | (109.80-130.23) |
| T4 |     | 9   | 6   | 5   | 0.717 |
|    |     | 210.00 | 137.72 | 130.23 |
|    |     | (181.40-220.00) | (124.49-155.00) | (98.37-146.82) |
| P-value |     | 0.233 | 0.089 | 0.730 | 0.035 |
| Negative |     | 162.70 | 122.00  | 112.14  |
|    |     | (123.82-208.05) | (107.60-140.54) | (98.69-123.71) |
| Positive |     | 146.35 | 119.50 | 123.23 |
|    |     | (125.28-179.70) | (100.00-129.00) | (110.95-137.08) |
| P-value |     | 0.509 | 0.646 | 0.179 | 0.001 |
| M |     | 159.18 | 121.27  | 115.68  |
|    |     | (125.90-193.02) | (105.23-129.42) | (98.37-136.70) |
| Positive |     | 158.27 | 123.00 | 113.45 |
|    |     | (117.85-207.52) | (107.60-140.54) | (110.83-130.23) |
| P-value |     | 0.893 | 0.748 | 0.785 | 0.038 |
| Response to FP therapy |     | 148.84 | 119.30  | 112.10  |
|    |     | (126.74-213.93) | (106.54-150.00) | (99.00-130.23) |
| No event of recurrence or progression |     | 160.40 | 123.50 | 131.91 |
|    |     | (119.32-190.06) | (117.00-136.00) | (109.83-137.45) |
| Recurrence |     | 125.06 | 101.30 | 107.34 |
|    |     | (113.95-134.00) | (97.41-118.71) | (95.82-151.09) |
| Progression |     | 0.250 | 0.054 | 0.587 | 0.846 |

Data presented as medians and IQR of global acetylated H3 %. * is a significant difference from baseline H3 acetylation, P ≤ 0.05.

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5711
Table 3: Global H4 acetylation in subgroups of CRC patients at baseline and after treatment with FP therapy

| Patients Subgroups                  | Global H4 acetylation % at baseline | Global H4 acetylation % after 3 months of FP therapy | Global H4 acetylation % after 6 months of FP therapy | P-Value |
|-------------------------------------|-------------------------------------|-----------------------------------------------------|-----------------------------------------------------|---------|
|                                     | N Median (IQR)                      | N Median (IQR)                                      | N Median (IQR)                                      |         |
| **Age**                             |                                    |                                                     |                                                     |         |
| ≤45                                 | 31                                  | 20                                                  | 13                                                  | 0.368   |
|                                     | (87.00-130.00)                      | (49.05-69.00)                                       | (42.34-60.15)                                       |         |
| >45                                 | 35                                  | 28                                                  | 19                                                  | 0.001   |
|                                     | (95.19-125.00)                      | (50.67-68.93)                                       | (38.45-44.42)                                       |         |
| **Sex**                             |                                    |                                                     |                                                     |         |
| Female                              | 30                                  | 26                                                  | 19                                                  | 0.005   |
|                                     | (84.02-111.45)                      | (50.54-55.00)                                       | (41.84-51.20)                                       |         |
| Male                                | 36                                  | 22                                                  | 13                                                  | 0.051   |
|                                     | (88.20-125.88)                      | (50.48-60.86)                                       | (37.45-47.65)                                       |         |
| **Baseline CEA**                    |                                    |                                                     |                                                     |         |
| Normal                              | 30                                  | 25                                                  | 17                                                  | 0.001   |
|                                     | (86.00-131.07)                      | (48.64-52.56)                                       | (43.05-59.55)                                       |         |
| High                                | 17                                  | 12                                                  | 7                                                   | 0.651   |
|                                     | (109.06-126.00)                     | (47.29-51.98)                                       | (40.55-51.20)                                       |         |
| **Baseline CA19.9**                 |                                    |                                                     |                                                     |         |
| Normal                              | 60                                  | 30                                                  | 11                                                  | 0.001   |
|                                     | (84.51-109.98)                      | (49.75-65.05)                                       | (40.93-60.15)                                       |         |
| High                                | 6                                   | 3                                                   | 3                                                   | 0.368   |
|                                     | (110.47-136.70)                     | (48.64-51.98)                                       | (99.24-120.24)                                      |         |
| **Site of tumor**                   |                                    |                                                     |                                                     |         |
| Right Colon                         | 47                                  | 33                                                  | 22                                                  | 0.001   |
|                                     | (86.40-126.00)                      | (49.61-53.17)                                       | (38.28-42.98)                                       |         |

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Table 3 continued

| Tumor Site          | Median H4% (IQR) | P-value |
|---------------------|------------------|---------|
| Colorectal cancer   |                  |         |
| T                   |                  |         |
| T1                  | 87.00 (64.10-120.00) | <0.05  |
| T2                  | 50.79 (46.00-60.60)  | 0.002   |
| T3                  | 78.80 (58.66-98.24)  | 0.003   |
| T4                  | 50.87 (49.05-54.00)  | 0.06    |
| LN                  |                  |         |
| LN Negative         | 86.20 (63.20-121.20) | <0.05  |
| LN Positive         | 88.20 (72.20-128.00) | 0.053   |
| M                   |                  |         |
| M Negative          | 86.70 (63.30-126.00) | <0.05  |
| M Positive          | 97.11 (69.33-120.60) | 0.062   |
| Response to FP therapy |              |         |
| No event of recurrence or progression | 87.00 (66.00-110.90) | <0.05  |
| Recurrence          | 75.47 (63.20-130.00) | 0.513   |
| Progression         | 62.53 (56.00-109.06)  | 0.717   |

Data presented as medians and IQR of global acetylated H4%. * is a significant difference from baseline H4 acetylation, P ≤ 0.05. ** is a significant difference from H4 acetylation after 3 months of FP therapy. IQR: interquartile range, NA: not applicable, T: tumor burden, N: lymph node, M: metastasis.
Similarly, this study tracked the changes in global H3 and H4 acetylation levels with FP therapy in the peripheral blood of CRC patients. Acetylation levels were correlated with the clinicopathological characters and the survival of patients.

PATIENTS AND METHODS

Patients
This is a prospective study in which whole blood samples were withdrawn from baseline 66 CRC patients who enrolled in the National Cancer Institute, Cairo University during the period from February 2014 to December 2014. After 3 and 6 months during their course of FP therapy, 48 and 32 of those patients were sampled again. Ten whole blood samples were collected from matched healthy controls in sex and age (male: female ratio= 1:1.3 and age= 38± 15.2, P= 0.89). Mononucleated lymphocytic cell pellets were isolated, as described previously in Fouad et al. (2018).

Global Histones Extraction and Quantification
The lymphocytic cell pellet was suspended in triton extraction buffer (0.5% Triton in phosphate-buffered saline, 2mM phenylmethylsulfonyl fluoride and 0.02% NaN3), and lysed on ice for 10 minutes with gentle stirring. After centrifugation, cell lysate was transferred to a new vial, and the residual cells were resuspended in the extraction buffer (0.5N HCl + 10% glycerol) and incubated on ice for 30 minutes. The supernatant fraction was taken to a new vial, and eight volumes of acetone were added and left at −20 °C overnight. The Protein concentration was quantified in the remaining dry pellet by Coomassie protein assay kit following Bradford (1976) method.

Level of Global H3 and H4 Acetylation
The EpiQuik™ Total Histone H3 and H4 Acetylation Detection Fast Kits (Epigentek, Farmingdale, NY, USA) were used according to the manufacturer’ protocol. The global content of acetylated histones in CRC patients was calculated from the protein calibration curve in ng/ total histone protein. Also, the percentage (%) of histones acetylation in the subgroups of CRC patients was calculated normalized to the level of acetylated histones in healthy controls multiplied to 100. The patients with % equal or lower than (≤) the median % were considered hypoacetylated, while patients with % more than (> ) the median % were considered hyperacetylated.

Survival analysis
After three years of follow up, event-free survival (EFS) of the 66 patients were tested by Kaplan and Meier test of analysis. EFS was calculated from the date of resection or neoadjuvant therapy to the date of recurrence, progression or death, which occurred first. EFS for patients who neither progressed, relapsed, nor died, was censored at last assessment before the loss of follow-up. Multivariate COX proportion hazard model was used to determine the hazard ratio of recurrence or progression during the EFS time in the subgroups of patients.

Statistical analyses
IBM SPSS statistical package version 24 was used in data manipulation. Numeric data explored for normality using Kolmogrov- Smirnov test and Shapiro-Wilk test. As numerical data, results were summarized as 25 percentile, median and 75 percentile. Patients were stratified into subgroups according to their clinicopathological features. Krucssill Wallas was used to test the significance for more than two subgroups of numerical data, and then Mann- Whitney was conducted for pairwise testing. Friedman tested the effect of FP therapy on genes expression over time (3 and 6 months) in a certain subgroup of CRC patients, followed by pairwise comparison with Wilcoxon matched test. All P-values were two-sided, and P-values ≤ 0.05 were considered significant.

RESULTS
This study included 66 Egyptian CRC patients. Their clinical characteristics are presented in Table 1. It was shown that the mean age ± SD was 46.5 ± 13.92, while the median was 46 years ranging from 19 to 72 years. Nearly half of the patients (47%) were 45 years with slight male predominance. Normal initial CEA and CA19.9 levels were recorded in 74.2% and 90.9% of the patients, respectively. It was found that 71.2 % of the patients had their primary tumour located in the right side, while 28.8 % were with left-sided location and rectal. Two-thirds of our patients had T3 tumours (60.6 %) while T4 was found in 13.6 %. The examined lymph nodes were negative in 63.6 % of the patients. Sixteen patients presented with metastatic disease. The treatment protocol was mainly FP based therapy taken either single in 25 % or combined with oxaliplatin in 75 %.

Baseline global H3 hyperacetylation but H4 hypoacetylation in CRC patients.
Before treatment with FP therapy, global acetylated H3 was significantly hyperacetylated compared to healthy control (medians= 2.63 versus 1.65 ng/ total histone protein, P< 0.001). “ is a significant difference when CRC patients after 3 and 6 months of FP therapy were compared with their baseline level, P value ≤ 0.05 Figure 1a. However,
Table 4: EFS time for subgroups of CRC patients

| Patients Subgroups | N   | Median EFS time (months) and 95% confidence interval | P-value |
|--------------------|-----|----------------------------------------------------|---------|
| Total              | 66  | 22.23 (16.20-28.26)                                 | NA      |
| Age                |     |                                                    |         |
| ≤45                | 31  | 20.83 (10.10-31.50)                                 | 0.861   |
| >45                | 35  | 22.23 (11.98-32.48)                                 |         |
| Sex                |     |                                                    |         |
| Female             | 30  | 22.23 (13.13-31.38)                                 | 0.587   |
| Male               | 36  | 19.97 (7.74-32.13)                                 |         |
| Baseline CEA level |     |                                                    |         |
| Normal             | 49  | 25.88 (21.93-29.89)                                 | 0.005   |
| High               | 17  | 9.10 (0.80-17.36)                                  |         |
| Baseline CA19.9 level |   |                                                    |         |
| Normal             | 60  | 25.90 (14.87-38.91)                                 | 0.047   |
| High               | 6   | 18.83 (10.22-27.49)                                 |         |
| Site of tumor      |     |                                                    |         |
| Right colon        | 47  | 24.17 (15.03-33.35)                                 | 0.592   |
| Left colon and Rectum |   |                                                    |         |
| T                  |     |                                                    |         |
| T2                 | 17  | 23.96 (18.57-29.36)                                 | 0.052   |
| T3                 | 40  | 22.23 (10.45-34.01)                                 |         |
| T4                 | 9   | 11.00 (4.57-17.43)                                 |         |
| LN                 |     |                                                    |         |
| Negative           | 42  | 25.11 (20.58-29.64)                                 | 0.028   |
| Positive           | 24  | 14.57 (11.30-17.83)                                 |         |
| M                  |     |                                                    |         |
| Negative           | 50  | 27.98 (24.21-31.75)                                 | <0.001  |
| Positive           | 16  | 5.83 (0.79-10.86)                                   |         |
| >87%               | 33  | 24.17 (9.83-38.50)                                 |         |

Data presented as median time in months and its 95% CI of the EFS of subgroups of CRC patients. N: number of patients, EFS: event free survival, CI: confidence interval, CEA: Carcinoembryonic antigen, CA19.9: Carbohydrate antigen 19.9, T: tumor burden, N: lymph node, M: metastasis
global acetylated H4 showed significant hypocetylation compared to healthy control (medians = 1.96 versus 2.25 ng/total histone protein, P = 0.004). " is a significant difference when CRC patients after 3 and 6 months of FP therapy were compared with their baseline level, P value ≤ 0.05 Figure 2a.

**FP-therapy-induced global hypoacetylation in Subgroups of CRC patients.**

Global H3 acetylation was significantly reduced by 0.24 and 0.28 folds after 3 and 6 months of FP therapy, respectively Figure 1b. The reduction in the % of acetylated H3 with FP therapy was significant in patients > 45 years, females and patients with right colon tumours. Also, significant H3 hypoacetylation with FP therapy was associated patients with normal baseline levels of CEA and CA19.9, patients with negative lymph nodes, and negative metastasis, and patients without signs of recurrence or progression after three years of follow up Table 2.

After 3 and 6 months of FP therapy, H4 acetylation was significantly reduced by 0.42 and 0.54 folds, respectively Figure 2b. By the subgrouping of CRC patients, a significant reduction in H4 acetylation with FP therapy was observed in patients > 45 years, females and patients with right colon tumours. Also, H4 hypoacetylation was associated patients with an average baseline level of CEA and CA19.9, patients with negative lymph nodes and negative metastasis, and patients without signs of recurrence or progres-
As demonstrated in Table 3, significant superior median EFS time was associated with patients with normal baseline CEA (P= 0.005) and CA19.9 levels (P= 0.047), and those with negative lymph nodes (P= 0.028) and negative metastasis (P<0.001).

The median % of H3 acetylation in our 66 CRC patients was 160%, while it was 87% for H4 acetylation. Significant improved EFS was associated CRC with H3 hyperacetylation >160% (median time= 24.17 months versus 14.63 months, P= 0.033) Figure 3a, and H4 hyperacetylation>87% (median time=22.59 months versus 15.87 months, P= 0.031) Figure 3b. The hazard of recurrence or progression was significant in subgroups of patients with high baseline CEA level (HR= 3.38, 95% confidence interval=1.19- 9.66, P= 0.023), positive metastasis (HR= 1.16, 95% confidence interval= 1.08-1.32, P<0.001), and H4 hypoacetylation <87% (HR= 1.55, 95% confidence interval= 1.09- 2.19, P=0.014), as shown in Figure 4.

**DISCUSSION**

In our previous study, the treatment outcome of CRC patients with FP- based therapy was significantly
dependent on their baseline global DNA methylation level and their clinicopathological characteristics (Foud et al., 2018). In the same way, global histones H3 and H4 acetylation levels were determined at baseline and after treatment with FP- therapy, to be then correlated with the patients’ response to treatment.

In this study, the nuclear histone proteins of baseline CRC patients exhibited significant global H3 hyperacetylation but H4 hypoacetylation. Baseline hypoacetylation of both types of histones (H3 and H4) was associated with a worse prognosis. For H3 acetylation, the immunostaining and expression of acetylated H3 in Hashimoto et al. (2013) showed high scores of acetylation proportional to the depth of CRC tumour invasion, higher pathological stages and worse prognoses. Researchers correlated the hyperacetylation of H3 with breast carcinoma cells proliferation and drug resistance (Simpson et al., 2010; Toth et al., 2012). H3 acetylation at specific lysine residues has shown to be increased in some and decrease in other cancers, implying that a functional interaction may exist between these two processes of hypo and hyperacetylation (Katan-Khaykovich, 2002). Ellinger et al. (2016) showed hypoacetylation of H3 in bladder cancer tissues. At the same time, Mosashvilli et al. (2010) recorded no change in acetylated H3 and H4 immunohistochemical staining among the diverse histological subtypes of renal cell carcinoma or oncocytoma samples. Suggesting that global level of histone acetylation does not depend only on balance between the enzymatic activities of HAT and HDAC but also on the intracellular acetyl-CoA pool (Ashktorab et al., 2009), on the type of cancer (Samec et al., 2019), and the levels of histones transcription-related factors (Sterner and Berger, 2000).

The negative prognostic significance of H4 hypoacetylation observed in this study was in line with many studies which correlate global histones hypoacetylation and its accompanied HDAC overexpression with the worse survival of CRC patients and a high chance of tumour recurrence (Chen et al., 2011; Benard et al., 2015). Moreover, the degree of H4 acetylation in oesophageal squamous cell carcinomas was shown to be dependent on tissue growth and depth of invasion (Karczmarski et al., 2014). A significant association was shown between the level of H4 acetylation and degree of metaplasia (Karczmarski et al., 2014). In gastrointestinal tumours, H4 was found to be hyperacetylated in the superficial parts of the tumour: In contrast, deeply invasive parts were hypoacetylated, suggesting that H4 hypoacetylation was not only involved in cancer development but also correlated with advanced tumour stage, invasion, and lymph node metastasis (Yasui et al., 2003).

The reversible nature of histones acetylation and their interaction with DNA and nuclear proteins has drawn the attention of the scientific community to study the molecular mechanism regulating its alteration, and the usefulness of using the epigenetic modifiers like HDAC inhibitors in anticancer therapy (Katan-Khaykovich, 2002; Khan, 2015).

In this study, both acetylated histones H3 and H4 were shown to be significantly reduced by FP therapy. The reduction of histones acetylation either globally or in specific lysine residues is a prominent carcinogenesis hallmark, in respect of elevated HDACs expression (Fraga et al., 2005). Du et al. (2017) and Liu et al. (2019) demonstrated increased resistance of CRC cells to 5-FU by the deacetylation of histones. 5FU reduced the binding ability of histone acetyltransferases (p300 and CBP) to chromatin and induced their degradation through lysosome. The expression level of p300/CBP was correlated with the clinical response to 5- FU therapy in CRC patients (Du et al., 2017). Also, 5- FU as DNA-damaging drug inhibits colon cancer cell growth and DNA repair by altering the regular cell cycle, creating its resistance state, which is associated with downregulation of histone acetyltransferase (PCAF), attenuating the p53-dependent transcription of p21, resulting in a subsequent increase in cyclin D1 and phosphorylation of retinoblastoma 1 (Liu et al., 2019).

Also, our data revealed FP- induced hypoacetylation associating CRC patients with good prognosis (normal baseline CEA and CA19.9 levels, negative lymph nodes and negative metastasis). Such observation could be correlated with the DNA damage response and DNA repair efficiency of cells toward the treatment with FP- therapy (Bracht et al., 2010). As known, CRC patients with deficient mismatch repair are highly associated with high-frequency microsatellite and better clinical outcome (Battaglin et al., 2018). However, their response to 5- FU exhibited resistance which was dependent on the DNA base excision repair pathway in Roos and Krumm (2016) and on Wnt pathway and checkpoint kinase 1 (CHK1) pathway in He et al. (2018) Chromatin Transcription (FACT) complex facilitates 5-FU repair in DNA via promoting the recruitment and acetylation of APE1 (AcAPE1) to the damage sites in chromatin (Song et al., 2020). Besides Chung et al. (2010) demonstrated the role of mismatch repair gene (hMLH1) dependency on SFU induced H3 hypoacetylation for DNA access promoting the resistance to cell death during the G1/S phase of
the cell cycle. Moreover, hypoacetylation created by HDAC was found to maintain the DNA-repair gene CtBP-interacting protein (CtIP) in its functional deacetylated state (Rajendran et al., 2013).

As seen in our results, other subgroups of CRC patients were subjected to FP- induced hypoacetylation. From them, elderly patients (> 45 years) showed significant hypoacetylation over the treatment period of FP- therapy. In concordance, Goossens-Beumer et al. (2015) demonstrated the different CRC epigenetic landscape in young versus elderly patients. Decreased expression of acetylated H3 with advancing age was observed in patients who were alive after follow-up (no-event group). In contrast, increased expression with advancing age was observed in patients who presented with a recurrence or death in follow-up (event group) Goossens-Beumer et al. (2015). Also, in this study, female patients were subjected to have more significant FP- induced hypoacetylation than male patients. Weisenberger et al. (2015) demonstrated the link of female sex with higher methylator phenotype, BRAF mutations, high microsatellite instability and promoter silencing to DNA repair gene (MLH1) associated with the absence of TP53 (Weisenberger et al., 2015). Also, better prognosis and more prolonged overall survival were observed in women compared to men related to specific genetic polymorphism in clock-genes, and higher levels of their regulating microRNA (miR-192, miR-206, miR-194, and miR-219) (Garufi et al., 2016). Moreover, our results showed that the FP- induced hypoacetylation was significant in patients with right-sided tumours than left-sided and rectal tumours. Such a difference was also observed in the global methylation pattern (Fouad et al., 2018), where right-sided colon cancers had higher rates of microsatellite instability, more frequent aberrant activation of the epidermal growth factor receptor pathway, and increased mutational burden compared to left-sided colon and rectal cancers (Salem et al., 2017). Making left-sided CRC patients benefit more from adjuvant chemotherapies such as 5-FU-based regimes (Baran et al., 2018).

CONCLUSION

In conclusion, nearly half of our patients were ≤ 45 years with slight male predominance. Almost two-thirds of our patients had T3, right-sided colon tumours with negative lymph nodes. The treatment protocol was mainly 5- fluorouracil based therapy either as a single agent or combined with oxaliplatin. Before treatment, chemonaive CRC patients showed global H3 hyperacetylation by 160%, but H4 hypoacetylation by 87% compared with healthy control. FP therapy significantly reduced global H3 and H4 acetylation levels, especially in CRC patients > 45 years, females and patients with right colon tumours. Also, significant hypoacetylation with FP therapy was associated patients with normal baseline levels of CEA and CA19.9, patients with negative lymph nodes, negative metastasis, and patients without signs of recurrence or progression after three years of follow up. Significant improved EFS was associated CRC with H3 hyperacetylation, while reduced EFS time and significant hazard of recurrence and progression were related to patients with high CEA, positive metastasis and H4 hypoacetylation.

ABBREVIATIONS

CRC: colorectal cancer, FP: fluoropyrimidine, H3: histone type 3, H4: histone type 4.
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