OPEN ACCESS

Genetic Study of Chemokine Ligand 1 in Colorectal Carcinoma using Quantitative Real-Time PCR

Athraa Alshimerry*, Dalia Amer Khudhair, Roaa Salih Mahdi

Department of Pathology, College of Medicine, University of Babylon, Babylon, Iraq

Abstract

BACKGROUND: Colon carcinoma is one of the prevalent carcinomas in the world and it is the third cause of cancer-related death in Western countries. The disease process is multifactorial; with etiology including inflammatory conditions of the digestive tract, environmental exposure, and genetic factors. Chemokine ligand 1 was shared in several mechanisms such as inflammatory process, chemoattraction, and others.

AIM: The present study was conducted to analyze the gene expression level of chemokine ligand 1 in colonic carcinoma and to deliberate its participation as genetic factors in its evolving and prognosis.

MATERIAL AND METHOD: *CXCL1* was evaluated in formalin-fixed, paraffin-embedded tissue blocks that were retrospectively collected from 40 patients (eight women and 32 men) with colonic carcinoma and 40 patients of normal colonic tissues as control specimens using real-Time PCR.

RESULTS: The expression of *CXCL1* was established as 12.4112-fold in carcinoma specimen about control tissue (1.3492). Chemokine ligand 1 genes were found to be overexpressed in advanced stage tumors and elderly patients.

CONCLUSION: Chemokine ligand 1 can be considered as a recent biomarker of colonic and colorectal carcinomas and a possible therapeutic target in the treatment of colonic carcinoma.

Introduction

Carcinoma of the colon is one of the most common malignancies and constitutes the third major reason for death due to cancer worldwide [1]. In addition, early diagnosis and molecular characterization are considered essential to decrease colonic carcinoma-related deaths [2]. The 5-year survival rate after curative surgery ranges between 40% and 60% [3]. Nowadays, there are determinate criteria both clinically and pathologically that are usually utilized in choosing the person who is suitable for adjuvant systemic therapy after surgery [4]. Therefore, the definition of prognostic markers in colonic carcinoma is very important in enhancing its prognosis [5]. Several factors either genetically (mutation of P53, KRAS, BRAF, and others genes) or environmentally are involved in the pathogenesis of colorectal carcinoma. The latter is largely dietary, particularly in consumption of a diet rich in fats and animal protein; because they influenced the microflora of the intestine and eventually on the chemical composition of the intraluminal content [3]. Among different factors, chemokines have a prevalent role in the pathogenesis of colonic carcinoma [6]. Chemokine is a small-sized protein manifested in different cells (leukocytes, epithelial cells, endothelial cells, and fibroblasts), as well as tumor cells [7], [8]. Usually, chemokines are divided into four large categories: C, CC, CXC, and CX3C, depending on the location of their cysteine residues [9]. A previous study revealed that chemokine ligand 1 is overexpressed in some types of human cancer, such as hepatocellular, bladder, prostate, skin cancers, and colorectal carcinoma [10], [11], [12], [13], [14]. *CXCL1* plays an axial role in the immune response of the host for bacterial killing by reactivating and recruiting neutrophils at the tissue site [15]. The aim of study is to determine the level of *CXCL1* mRNA expression in colonic tumors and to study its correlation with clinic pathological parameters in Iraqi patients.

Materials and Methods

This retrospective study was approved ethically and was carried out in the Department of Pathology, College of Medicine – University of Babylon, Iraq, from January 2017 to August 2019 and the specimens were collected from some general hospitals and some private laboratories in Hila city.

Eighty formalin-fixed, paraffin-embedded tissue blocks were included in this study. Forty blocks of normal colonic tissues were used to normalize data and 40 blocks (32 men and eight women) with colonic carcinoma, their ages were ranging from 32 to 79 years. Grading of the presented malignant cases was assessed according to the WHO grading system, showing that 22 cases were well-differentiated carcinoma, eight cases were moderately differentiated carcinoma, and 10 cases were poorly differentiated carcinoma. The staging of colonic carcinoma according to the TNM staging system showed that T1 was 11 cases T1, T2 was 10 cases, and T3 was 19 cases [3].

Extraction of RNA

Using (TRIzol® reagent kit, AccuZo1™, Bioneer, Korea), total ribonucleic acid (RNA) was extracted from tissue specimens. Afterward, the DNase I enzyme kit was utilized to remove the amounts of genomic DNA. Then, the RNA integrity number (RIN) was assessed by determined the purity of RNA by reading the ratio of absorbance at A260 and A 280 in Nanodrop spectrophotometer. The absorption maximum of RNA is at 260. If the ratio was 1.8–2.0, it indicates that the purity of RNA was in the optimal value (our result was 2.0).

Complementary DNA (cDNA) synthesis

A 100 ng/ul of extracted RNA was reverse-transcribed using M-MLV reverse transcriptase kit (AccuPower® RocketScript™ RT PreMix, Bioneer, Korea). Reverse transcriptase enzyme synthesizes a single-stranded DNA in the presence of primers and using mRNA molecule as a template, the primer oligo dT (annealing to poly A tails of mRNA).

Quantitative real-time PCR (qPCR)

Using specific primers for *CXCL1*, the first newly synthesized strand of cDNA was subjected to a real-time polymerase chain reaction. A 5 µL cDNA, 25 µL 2X GreenStar Master Mix, 2 µmol/L forward primers, and 2 µmol/L reverse primers, 16 DEPC water were added to AccuPower™ 2X GreenStar qPCR Master Mix kit (Bioneer, Korea). The reaction conditions were as follows: Initial denaturation at 50°C for 1 h for 1 cycle, then 40 cycles at 95°C for 20 s, and then 40 cycles of 60°C for 0.5 min, last, 1 cycle of 60–95°C for 0.5 s. The 5′-CCAAAGTGTGAACGTGAAGTCC-3′ sequence is for the forward primer of CXCL1 and that of the reverse primer was 5′- AAGCTTTCCGCCCATTCTTG-3′, and that of a housekeeping gene (GAPDH) was 5′- AATTCCATGGCACCGTCAAG-3′ (forward) and 5′-ATCGCCCCACTTGATTTTGG-3′ (reverse); (Bioneer company, Korea). The gene expression analysis of CXCL1 to GAPDH was accounted just as the average $2-\Delta Ct$ where ΔCt (cycle threshold) = Ct – CtGAPDH.

Statistical analyses

Using two software programs, statistical analysis was performed. These programs were (SPSS version 18) using t-test one-way ANOVA test, Chi-square test (p value significance level < 0.05), and Microsoft Office Excel 2007.

Results

Forty cases of colonic carcinoma and 40 cases of normal colonic tissue were comprised in the present study (Table 1). The clinicopathological assessment of colonic carcinoma cases disclosed that 32 (80%) patients were men and 8 (20%) were women, with a difference between men and women being highly significant (p < 0.05) (Table 1).

Table 1: The clinicopathological characteristics of the study samples

Parameter	No. of cases	Percentage	p value
Types of tissue			
Normal colonic (control group) tissue	40	50%	<0.05*
Colonic carcinoma (study group)	40	50%	
Gender			
Male	32	80%	
Female	8	20%	<0.05*
Age			
<50	14	35%	
>50	26	65%	<0.05*
Grade			
Well-differentiated	22	55%	
Moderately differentiated	8	20%	
Poorly differentiated	10	25%	<0.05*
Stage			
T1	11	27.5%	
T2	10	25%	
T3	19	47.5%	<0.05*

*The mean difference is significant at the 0.05 level.

According to the age, the cases were classified into two groups. The first group consisted of 14 (35%) samples of patients of ages ≤50 years, while the second group consists of 26 (65%) samples of patients who have ages >50 years. According to the independent t-test, the difference between these two age groups is significant (p < 0.005) (Table 1).

Assessment of grade shows that well-differentiated tumor was reported in 22 (55%) cases, while the moderately differentiated tumor was 8 (20%) cases and poorly differentiated tumors were 10 (25%) with significant difference between these grades p < 0.05 (Table 1).

Assessment of the stage (T) of the 40 cases of colonic carcinoma showed that 11 (27.5%) cases were of T1, 10 (25%) of T2, and 19 (47.5%) of T3, the difference in the frequency between these stages is significant (p < 0.05) (Table 1).

CXCL1 mRNA (fold change) was found to be 1.35 in normal colonic and 12.39 in malignant tissue, so according to independent t-test, *CXCL1* gene
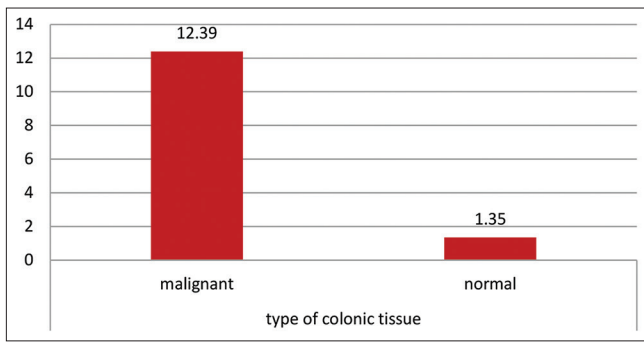
Figure 1: CXCL1 gene expression level (mean fold change) in non-tumorous tissue and colorectal carcinoma

expression significantly (p < 0.0001) raised in colorectal carcinoma relative to normal colonic tissues (Figure 1).

In this study, the one-way ANOVA test was performed to compare mean fold change between gender in which the mean fold change of the females cases was 9.172 and was 13.192 for males (Figure 2) with no significant difference between these two groups, p = 0.189.
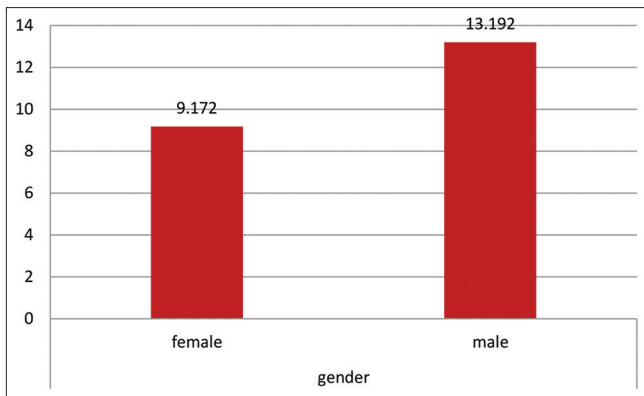

Figure 2: CXCL1 gene expression level (mean fold change) about gender

According to the ages of colonic carcinoma samples, *CXCL1* gene expression will be explored and the one-way ANOVA test analysis was used to evaluate the data. The mean fold change was 3.988 for the age group ≤50 years old and was 15.615 for the age group > 50 years old with highly significant difference between these fold changes (p = 0.0001) (Figure 3).
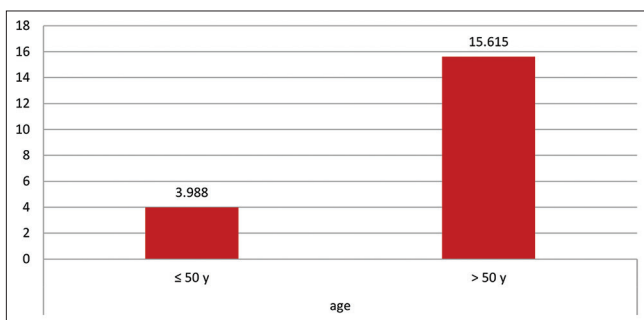

Figure 3: CXCL1 gene expression level (mean fold change) about age

According to the one-way ANOVA test, the difference between moderately differentiated and the

well-differentiated tumor is significant (p = 0.0001) and between poorly differentiated and the well-differentiated tumor is also significant (p = 0.0001) but the difference between poorly and moderately differentiated is not significant (p = 0.51) in which the mean of well-differentiated tumor was 18.785, moderately differentiated tumor was 6.288, and poorly differentiated tumor was 2.546 (Figure 4a-c).
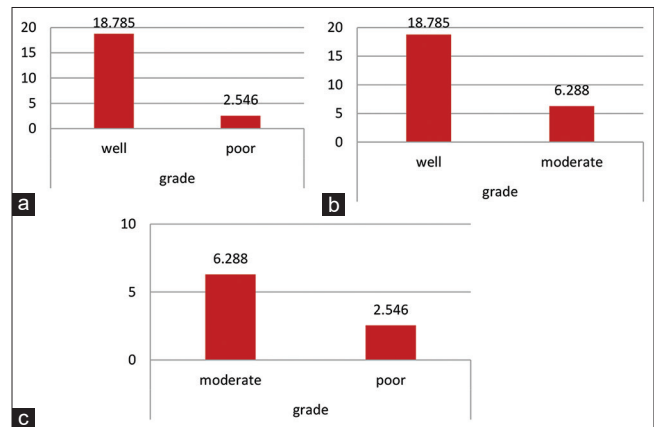

Figure 4: CXCL1 gene expression level (mean fold change) about the grade

According to the one-way ANOVA test, there is a significant difference between Stage T2 and Stage T1 (p = 0.0001) and between T3 and T1 (p = 0.0001), also, there is a significant difference between T3 and T2 (p = 0.0001) in which the mean of T1 was 2.871, T2 was 10.133, and T3 was 19.067 (Figure 5a-c).
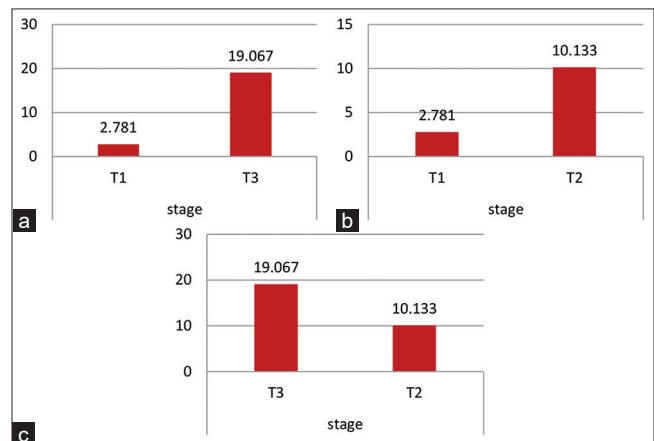

Figure 5: CXCL1 gene expression level (mean fold change) about the stage

Discussion

Obviously, many researchers have been focusing on the understanding the biology of colonic carcinoma. Early metastatic pathological signs include invasion to a blood vessel, lymphatic vessel invasion, or multiple presentations [16]. Nowadays, the chemokine

family is receipting high interest as multifunctional proteins [17]. Dysregulated genes have been determined in malignant tissue by a genome broad method and these genes can be used for staging of malignant tumor and can be directed in the strategies of treatment [18], [19], [20]. The previous studies have identified an overexpression of chemokine ligand L1 in ulcerative colitis and colon cancer of human beings [18], [21], [22], [23], [24]. Here, we estimated the level of *CXCL1* mRNA expression in colonic tissue by real-time PCR and we found a highly significant difference (p < 0.0001) between cancer tissues and normal colonic tissue in which the expression level was 12.4112-fold in colonic cancer and was 1.35 in normal colonic tissues (Figure 1). Such results suggested the upregulation of the expression of *CXCL1* gene during carcinogenesis. Findings of the upregulation of the *CXCL1* expression in colorectal carcinoma patients are in concordance with the data of Zhuo *et al.,* 2018 [25]. The present study shows no significant difference between males and females (P=0.189), in spite the mean fold change of *CXCL1* is more in males (13.192) than in females (9.172) (Figure 2), this study is in concordance with Zhuo *et al.,* 2018 [25], but the difference is highly significant between two age groups (p = 0001) in which *CXCL1* mRNA was 15.615 in the age group of more than 50 years and was 3.988 for those equal or less than 50 (Figure 3), this result was disagreement with Zhuo *et al.,* 2018 [25]. Furthermore, in this study, the relationship between tumor grade and *CXCL1* mRNA expression was examined and showed significant difference (p = 0.0001) between moderately differentiated and well-differentiated tumors and between poorly differentiated and well-differentiated tumor the difference is also significant (p = 0.0001) but the difference between poorly and moderately differentiated is not significant (p = 0.51) in which the expression level (fold change) was very high in well-differentiated tumor (18.785) (Figure 4), while the Zhuo *et al.,* 2018 [25], was found that high fold change was in the moderately differentiated tumor. A significant (p = 0.0001) rise of the *CXCL1* gene expression fold was observed in those of Stage T3 when they were compared with those of Stage T1. Similarly *CXCL1* gene expression was evident to be increased significantly (p = 0.0001) in tumor of Stage T3 concerning those of Stage T2 (Figure 5). In the present study, *CXCL1* expressions were found to elevate as the stages were advanced. These results are consistent with the previous reports Zhuo *et al.,* 2018 [25], that have pointed out a significant correlation of *CXCL1* expression with advancing colonic carcinoma stages.

Conclusion

This study proposes that highly elevated *CXCL1* expression can enhance tumor formation and can be used as a poor prognostic marker in the advanced age of colonic carcinoma patients and advanced-stage patients and can be used as a potential therapeutic target in the treatment of colorectal carcinoma.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2018;68(1):7-30. https://doi.org/10.3322/caac.21442
   PMid:29313949

2. Heurta S. Recent advances in the molecular diagnosis and prognosis of colorectal cancer. Expert Rev Mol Diagn. 2008;8(3):277-88. https://doi.org/10.1586/14737159.8.3.277
   PMid:18598107

3. Rosia J. Rosai and Ackerman's Surgical Pathology. 10th ed., Vol. 17. Campus Outreach St. Louis. CV Mosby Year Book Inc.; 2011. p. 1247-86. Available from: https://www.elsevier.com/books/rosai-and-ackermans-surgical-pathology-10e/rosai/978-81-312-2984-2. [Last accessed on 2021 Dec 15]. https://doi.org/10.1016/s0046-8177(04)00455-1

4. Allegra CJ, Paik S, Colangelo LH, Parr AL, Kirsch I, Kim G, *et al.* Prognostic value of thymidylate synthase, Ki-67, and p53 in patients with dukes' B and C colon cancer: A national cancer institute-national surgical adjuvant breast and bowel project collaborative study. J Clin Oncol. 2003;21(2):241-50. https://doi.org/10.1200/jco.2003.05.044
   PMid:12525515

5. Oladipo O, Conlon S, O'Grady A, Purcell C, Wilson C, Maxwell PJ, *et al.* The expression and prognostic impact of CXC-chemokines in stage II and III colorectal cancer epithelial and stromal tissue. Br J Cancer. 2011;104(3):480-7. https://doi.org/10.1038/sj.bjc.6606055
   PMid:21285972

6. Emmanouil G, Ayiomamitis G, Zizi-Sermpetzoglou A, Tzardi M, Moursellas A, Voumvouraki A, *et al.* Angiodrastic chemokines in colorectal cancer: Clinicopathological correlations. Anal Cell Pathol (Amst). 2018;2018:1616973. https://doi.org/10.1155/2018/1616973
   PMid:29850390

7. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7-30. https://doi.org/10.3322/caac.21332
   PMid:6742998

8. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin. 2007;57(1):43-66. https://doi.org/10.3322/canjclin.57.1.43
   PMid:17237035

9. Monteagudo C, Pellín-Carcelén A, Martín JM, Ramos D. Role of chemokines in melanoma progression. Actas Dermosifiliogr. 2011;102(7):498-504. https://doi.org/10.1016/j.ad.2011.03.004
   PMid:21531362

10. Liu Z, Yang L, Xu J, Zhang X, Wang B. Enhanced expression and clinical significance of chemokine receptor CXCR2 in hepatocellular carcinoma. J Surg Res. 2011;166(2):241-6. https://doi.org/10.1016/j.jss.2009.07.014
    PMid:20018298

11. Miyake M, Lawton A, Goodison S, Urquidi V, Rosser CJ. Chemokine (C-X-C motif) ligand 1 (CXCL1) protein expression is increased in high-grade prostate cancer. Pathol Res Pract. 2014;210(2):74-8. https://doi.org/10.1016/j.prp.2013.08.013
    PMid:24252309

12. Lai TH, Wu PH, Wu WB. Involvement of NADPH oxidase and NF-κB activation in CXCL1 induction by vascular endothelial growth factor in human endometrial epithelial cells of patients with adenomyosis. J Reprod Immunol. 2016;118:61-9. https://doi.org/10.1016/j.jri.2016.08.011
PMid:27665197

13. Miyake M, Lawton A, Goodison S, Urquidi V, Gomes-Giacoia E, Zhang G, *et al*. Chemokine (C-X-C) ligand 1 (CXCL1) protein expression is increased in aggressive bladder cancers. BMC Cancer. 2013;13:322. https://doi.org/10.1186/1471-2407-13-322

14. Verbeke H, Struyf S, Laureys G, Van Damme J. The expression and role of CXC chemokines in colorectal cancer. Cytokine Growth Factor Rev. 2011;22(5-6):345-58. https://doi.org/10.1016/j.cytogfr.2011.09.002
PMid:22000992

15. Sawant KV, Poluri KM, Dutta AK, Sepuru KM, Troshkina A, Garofalo RP, *et al*. Chemokine CXCL1 mediated neutrophil recruitment: Role of glycosaminoglycan interactions. Sci Rep. 2016;6:33123. https://doi.org/10.1038/srep33123
PMid:27625115

16. Koelzer VH, Lugli A, Dawson H, Hädrich M, Berger MD, Borner M, *et al.* CD8/CD45RO T-cell infiltration in endoscopic biopsies of colorectal cancer predicts nodal metastasis and survival. J Transl Med. 2014;12:81. https://doi.org/10.1186/1479-5876-12-81
PMid:24679169

17. Kuo PL, Shen KH, Hung SH, Hsu YL. CXCL1/GROα increases cell migration and invasion of prostate cancer by decreasing fibulin-1 expression through NF κB/HDAC1 epigenetic regulation. Carcinogenesis. 2012;33(2):2477-87. https://doi.org/10.1093/carcin/bgs299
PMid:23027620

18. Carvalho B, Sillars-Hardebol AH, Postma C, Mongera S, Terhaar Sive Droste J, Obulkasim A, *et al.* Colorectal adenoma to carcinoma progression is accompanied by changes in gene expression associated with ageing, chromosomal instability, and fatty acid metabolism. Cell Oncol (Dordr). 2012;35(1):53-63. https://doi.org/10.1007/s13402-011-0065-1
PMid:22278361

19. Xu JZ, Wong CW. Hunting for robust gene signature from cancer profiling data: Sources of variability, different interpretations, and recent methodological developments. Cancer Lett. 2010;296(1):9-16. https://doi.org/10.1016/j.canlet.2010.05.008

20. Velenik V, Ocvirk J, Oblak I, Anderluh F. A phase II study of cetuximab, capecitabine and radiotherapy in neoadjuvant treatment of patients with locally advanced resectable rectal cancer. Eur J Surg Oncol. 2010;36(3):244-50. https://doi.org/10.1016/j.ejso.2009.12.002
PMid:20042310

21. Wang D, Wang H, Brown J, Daikoku T, Ning W, Shi Q, *et al.* CXCL1 induced by prostaglandin E2 promotes angiogenesis in colorectal cancer. J Exp Med. 2006;203(4):941-51. https://doi.org/10.1084/jem.20052124
PMid:16567391

22. Martyna B, Małgorzata MW, Nikola Z, Beniamin G, Urszula M, Grażyna J. Expression profile of genes associated with the proteins degradation pathways in colorectal adenocarcinoma. Curr Pharm Biotechnol. 2019;20(7):551-61. https://doi.org/10.2174/1389201020666190516090744
PMid:31096896

23. Kita H, Hikichi Y, Hikami K, Tsuneyama K, Cui ZG, Osawa H, *et al.* Differential gene expression between flat adenoma and normal mucosa in the colon in a microarray analysis. J Gastroenterol. 2006;41(11):1053-63. https://doi.org/10.1007/s00535-006-1894-y
PMid:17160516

24. Sillars-Hardebol AH, Carvalho B, De Wit M, Postma C, Delis-van Diemen PM, Mongera S, *et al.* Identification of key genes for carcinogenic pathways associated with colorectal adenoma-to-carcinoma progression. Tumour Biol. 2010;31(2):89-96. https://doi.org/10.1007/s13277-009-0012-1
PMid:20358421

25. Zhuo C, Wu X, Li J, Hu D, Jian J, Chen C, *et al.* Chemokine (C-X-C Motif) Ligand 1 (CXCL1) is associated with tumorprogression and poor prognosis in patients with colorectal cancer. Biosci Rep. 2018;38(4):580. https://doi.org/10.1042/bsr20180580
PMid:29784873