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THE STUDY OF BCL-2 C938A GENE POLYMORPHISM IS PROMISING FOR ASSESSING THE RISK OF OCCURRENCE AND/OR EARLY DIAGNOSIS OF COLORECTAL CANCER

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ABSTRACT

Molecular-genetic study of the Bcl-2 C938A gene polymorphism in patients with colorectal cancer in age and gender aspects. Blood samples of 48 patients were tested for the presence of the Bcl-2 C938A gene polymorphism using the AC-PCR method. The genotype and allele frequency of Bcl-2 SNP (-938C>A) were statistically analyzed. The predominance of the heterozygous variant of the CA genotype was established in both men and women diagnosed with colon adenocarcinoma. The homozygous variant of the CC genotype did not have statistically significant difference in the comparison groups. In a group of practically healthy women, a homozygous variant of the AA genotype was detected with a frequency of 80.0%. The heterozygous variant of the CA genotype of the C938A polymorphic variant of the Bcl-2 gene is statistically significantly associated with colon cancer in both men and women. Determination of Bcl-2 gene polymorphism at position 938 can become a marker for early diagnosis of colorectal cancer and can also be a promising criterion for assessing the risk of neoplastic lesions of the colon.

1. Introduction

DNA repair processes are closely and inextricably linked to the cell cycle control system and apoptosis, since single- or double-strand breaks in DNA can prevent normal cell division or cause point mutations and chromosomal aberrations in the generation of the damaged cell.

DNA repair systems, cell cycle control and apoptosis play a decisive role both in the formation of the body's response to the influence of adverse exogenous or endogenous factors, and in the development of distant consequences of these influences.

Due to DNA repair and cell cycle delay the damage to the genetic material is eliminated because of a direct damaging effect on the target cell and induced by further instability of its genome in the offspring cells. The actual process of apoptosis allows eliminating cells with irreversible changes in the genome from the body. Together, the process of these systems allows preserving the genetic integrity of the body and preventing the appearance of cells with significant functional disorders and oncogenic potential [1].

Systems supporting the integrity of the genome are represented by various protein complexes that interact with each other and with other structures through branching cascades of reactions.

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The genes that encode these proteins are significantly conserved and any major changes in their coding or regulatory loci are usually screened out of the gene pool.

However, it should be noted that point mutations SNPs (single-nucleotide genetic polymorphisms), which under normal conditions of the organism's existence did not have a significant impact on the functioning of these systems, were fixed in the population during the evolution process. Nevertheless, in the presence of atypical damaging factors in amounts that significantly exceed the natural background, these polymorphic variants in the genes of the DNA repair, cell cycle control, and apoptosis systems can modify the body's normal response to endo- or exogenous traumatic factors and significantly influence the development of negative long-term consequences.

The Bcl-2 gene is a negative regulator of apoptosis. The special interest in the C938A polymorphism of the Bcl-2 gene is determined by its proximity to the DNA region to which the p53 protein binds (p53-response element), which plays a key role in maintaining genome stability and preventing the duplication of damaged DNA [2].

Nowadays, the international scientific literature has accumulated significant data directly or indirectly confirming that there is a physiological connection between gene polymorphisms of DNA repair systems, cell cycle control, apoptosis with effects such as a high level of mutations in the TP53 gene and an increased level of spontaneous and induced chromosomal aberrations.

There are also confirmed connections of these polymorphisms with increased risk of oncopathology of various types and locations, reduced life expectancy, low efficiency and/or poor tolerance to chemotherapy and radiation therapy [3].

Bcl-2 was the first gene identified to prevent cell death, which has an important influence on tumor biology.

Several members of the human Bcl-2 proteins family capable of regulating apoptosis have been identified, including three antiapoptotic, three structurally similar proapoptotic proteins, and several structurally diverse proapoptotic interacting proteins acting either as agonists or as antagonists.

A large number of post-translational modifications and interactions with other proteins, in turn, regulate these proteins.

There is significant evidence that the regulation of genes encoding either anti-apoptotic or pro-apoptotic proteins of the Bcl-2 family is altered in cancer diseases.

In addition to changes in Bcl-2 gene structure or copy number, many additional mechanisms contribute to increased gene expression, which is estimated to occur in nearly half of all human cancers.

The Bcl-2 (B-cell leukemia/lymphoma 2) gene has been shown to be associated with the development of breast cancer, and a single nucleotide polymorphism (SNP; -938C>A) has recently been identified [4, 5].

It was demonstrated that overexpression of Bcl-2 and related anti-apoptotic proteins that inhibit cell death, is induced by a variety of biological stimuli, including growth factor deprivation, hypoxia, and oxidative stress [6, 7].

Moreover, it was established that SNPs of the gene belonging to the Bcl-2 family play a significant role in chemotherapy resistance and evaluation of Bcl-2-938C>A and BAX-248G>A polymorphisms may be useful in predicting clinical outcomes in patients with advanced inoperable non-small cell lung cancer to platinum-based chemotherapy [8].

Considering the above-mentioned information, as well as to confirm the hypothesis about the promising study of SNPs of Bcl-2-938C>A as a possible marker for assessing the risk of neoplastic lesions of the colon and/or detecting CRC in its early stages, we conducted a study of the distribution of Bcl-2 C938A polymorphic variants in patients with colorectal cancer (non-terminal stages) considering the age and gender characteristics.

In this article, we show the results of studies of the Bcl-2 C938A gene polymorphism in a small cohort of patients. Unfortunately, the war dramatically changed not only our scientific plans, but also many other factors. We believe that we will be able to continue our research in the near future.

2. Material and methods

Study Patients and samples collection

Blood samples of 48 patients were tested for the presence of the Bcl-2 C938A gene polymorphism using the AC-PCR method. At the time of sample collection, all patients were receiving inpatient treatment at the Ternopil Regional Oncology Dispensary. There were 16 men and 12 women among CRC patients. The average age of men was (63.0 ± 2.8) years. The average age of women was (64.0 ± 2.7) years. The control group included 20 patients (10 women and 10 men) in whom no neoplastic lesions were detected during the examination.

The average age of men in the comparison group was (60.0 ± 3.4) years; women – (59.0 ± 3.6) years. The blood sample contained 2 ml, which was collected from patients' v. cubitalis, using a special vacuum system – vacutainer with 3% EDTA (Vacusera, Turkey). Hereafter, samples were turned upside down several times to mix blood with anticoagulant.

All patients gave written informed consent to participate in the study.

Molecular genetic study of the frequency of polymorphic variants of the Bcl-2 gene C-938A (rs2279115)

Genotyping of the SNP -938C>A polymorphism was determined using allele-specific polymerase chain reaction (AC-PCR). The principle of the method is based on an allele-specific reaction, which consists in matching the terminal nucleotide at the 3'-end of the primer with the sequence of the matrix of the identifying allele. If the 3'-end of the primer does not match with the matrix, the amplification reaction does not occur. As an amplification control, each tube was also given a pair of primers specific to the region of the albumin gene.

The primers composition was developed jointly by specialists of the Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine and PE "LPB "NEO-GEN". Primers were synthesized by Eurofins Genomics (Austria, ISO 13485 certified).

The following primers (10 pmol) were used to generate specific fragments:

forward GTCAATCCGCAGGAATCCCA
reverse CCGGCTCCTTCATCGTCCCC
reverse (replacing C with A) CCGGCTCCTTCATCGTCCCA
reverse (replacing C with T) CCGGCTCCTTCATCGTCCCT
DNA isolation and genotyping
Genomic DNA was isolated from the whole blood using a set of reagents NeoPrep100 DNA Magnet Blood (NEO-GEN laboratory, Ukraine).

The volume of the blood sample for selection was 50 µl.
The amplification cycle was as follows: initial sample denaturation at 95°C for 5 min; 4 cycles of sample amplification at 95°C for 15 sec, at 63°C for 15 sec, at 72°C for 15 sec, and a final elongation of the sample at 72oC for 2 minutes.
DNA detection was carried out by electrophoresis using a set of reagents from LPB NEO-GEN, Ukraine. Electrophoresis was performed at a voltage of 8 Volts per centimeter of 3% ethidium bromide added to agarose gel within 60 min. After electrophoresis, photographs were taken under an ultraviolet light transilluminator.

Statistical analysis
The main part of the statistical analysis was carried out using the Statistica 7.0 (SPSS) program. The genotype and allele frequency of Bcl-2 SNP (-938C>A) were tested using the public statistical web-tool http://www.oege.org/software/hwe-mr-calc.shtml for Hardy-Weinberg equilibrium (HWE). Reliability of differences in mean values for groups with different genotypes was determined using the one-factor method analysis of variance (one-way ANOVA). Statistical significance was set at $p < 0.05$.

3. Results

Treatment and prevention of cancer in Ukraine is extremely relevant. Due to the significant tendency in the incidence of cancer to increase, doctors need additional methods of controlling malignant tumors in the population. This is especially significant for colorectal cancer. In most cases, patients seek for specialized help in the late stages of the disease, which are incurable. One of the ways to solve this problem is the introduction of new methods for early detection of colorectal cancer into medical laboratory diagnostics. In our opinion, one of these methods can be the study of the Bcl-2 C938A gene polymorphism.

Patient characteristics
Patients' characteristics and genotypic data are shown in Table 1. In brief, all patients were Ukrainians, 26 (54.2 %) males and 22 (45.8 %) females at a median age of 61 year (range between 55 and 67 years old).
It was found that in male patients with adenocarcinoma of the large intestine, a statistically significant increase in the detection frequency of the CA genotype was established (68.75%) in comparison with a similar indicator in the control group patients (30.0%).
CC and AA genotypes in men with neoplastic lesions of the colon occurred with a frequency of 12.5% and 18.75%, respectively.
In men of the control group, the detection frequency of these genotypes was 50.0% and 20.0%, respectively (Figure 1).
In female patients with neoplastic lesions of the large intestine, the detection frequency of the CA genotype was 58.3%; genotype CC–25.0%; genotype AA– 16.7%.
In women of the control group (without signs of carcinogenesis), the CA genotype was not detected at all. 80.0% of women in this group had the homozygous AA genotype, 20.0% of practically healthy women had the homozygous CC genotype (Figure 2).
The conducted molecular genetic study of polymorphism of the Bcl-2 gene (C938A) made it possible to establish the predominance of the heterozygous variant of the CA genotype in both men and women diagnosed with colon adenocarcinoma. The homozygous variants of the CC and AA genotypes were not statistically different in the comparison groups.

Characteristics	**n**		**%**	
Age, years				
Median	61			
Range	55–67			
Gender				
Male	26		54.2	
Female	22		45.8	
Stage				
0 (Adenocarcinoma *in situ*)	18		64.3	
II (N_0M_0)	10		35.7	
Histologic type				
Adenocarcinoma	28		100.0	
Weight loss (experimental group)				
Yes	23		82.1	
No	5		17.9	
Bcl-2 -938C>A (male)	Control, n	CRC, n	Control, %	CRC, %
CC	5	2	50	12
CA	3	11	30	19
AA	2	3	20	69
Bcl-2 -938C>A (female)	Control, n	CRC, n	Control, %	CRC, %
CC	2	3	20	25
CA	0	7	0	58
AA	8	2	80	17

Table 1. Patients' characteristics (n = 28)

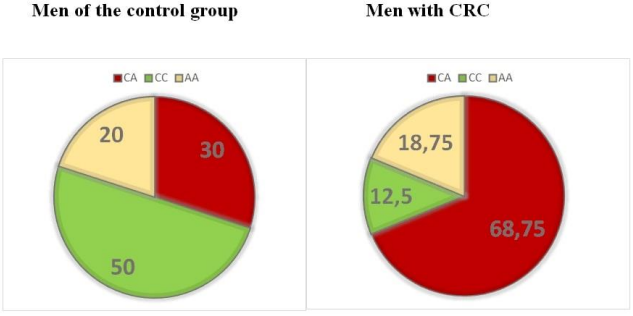

Figure 1. The frequency of CA, CC, and AA genotypes in male patients of the control and experimental groups (%).

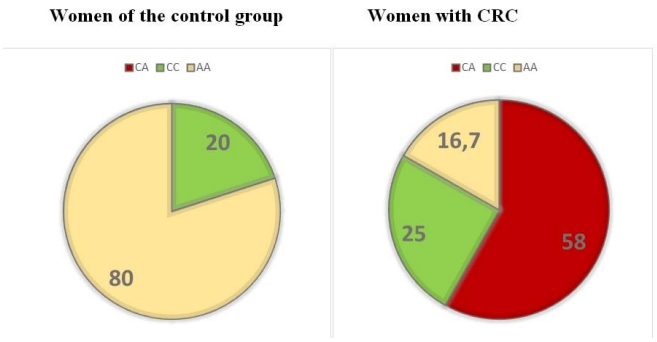

Figure 2. The frequency of different genotypes detection in women of the experimental and control groups (%).

4. Discussion

Apoptosis is a process of programmed cell death which occurs under both physiological and pathological conditions. Apoptosis is a mechanism to regulate homeostasis in the human body. The balance of anti-apoptotic and pro-apoptotic proteins regulates cell fate and controls the response to apoptotic signals [10, 11, 12].

Apoptosis is carried out by intracellular cysteine proteases – caspases. Approximately 10 caspases in mammalian cells are synthesized as mostly inactive zymogens, and two major pathways activate those involved in apoptosis. An older, evolutionarily conserved "stress" pathway, which is triggered primarily by various intracellular stresses, activates caspase-9 in response to the release of cytochrome C from damaged mitochondria. In this pathway, also called the "mitochondrial" or "intrinsic" pathway, the Bcl-2 protein family representatives are the central gatekeepers. The “extrinsic” pathway, on the other hand, is induced when so-called “death receptors” on the surface of cells are recruited by related ligands of the tumor necrosis factor (TNF) family. Instead, this pathway activates caspase-8 (and caspase-10 in humans) via adapter proteins that include the Fas-associated death domain protein (FADD).

Once activated, caspase-9 or -8 (-10) activates subsequent “effector caspases” (i.e., caspases-3, 6, and 7), which provoke cell destruction by cleaving several hundred cellular proteins. These two pathways are largely independent to prevent catastrophic unscheduled cell death, both tightly regulated in multiple steps [13].

Shortage in apoptosis alters intracellular homeostasis and might lead to tumorigenesis and stimulate tumor progression [14, 15, 16].

Anti-apoptotic Bcl-2 protein is a key regulator of the apoptotic process. Due to its widespread expression in various malignancies, it is considered as a predictive biomarker or therapeutic target in the diagnosis of cancer [17, 18, 19].

SNPs of Bcl-2 family genes are believed to play an important role in many aspects of carcinogenesis. It has been shown that the SNPs of the Bcl-2 gene affects the course of cancer, its consequences, and the result and effectiveness of the applied chemotherapy [8, 20].

At the same time, the results of the analysis of C938A Bcl-2 gene polymorphic variants in carcinogenesis, obtained by various researchers, are quite contradictory.

Nuckel H. et al. [21] has found that Bcl-2 protein expression in B-lymphocytes of patients with chronic lymphocytic leukemia carrying the -938 AA genotype was significantly increased compared with CC genotype. It has also been confirmed by other studies in prostate cancer [22], renal cancer [23], and breast cancer [24].

Zhang et al. [4] had the opposite result, which shows that the -938A allele contributes to the reduction of Bcl-2 protein expression in breast cancer cell lines. In addition, previous studies have shown that the BCL2-938 A variant was associated with a reduced risk of squamous cell carcinoma of the head and neck [25] and prostate cancer [26], but an increased risk of glioma [27].

Further research is needed to clarify these contradictions.

Colorectal cancer (CRC) is one of the most prevalent types of cancer, being the third most common type in the world [28]. Colorectal cancer is the result of many genetic occurrences.

Several pathways initiate the pathogenesis of CRC, but there are only 3 main ones: 1) the chromosomal instability pathway (CIN), the microsatellite instability pathway (MSI), and the serrated pathway. Of these, the CIN pathway is the main one. It is distinguished by a violation of chromosomal segregation, telomere stability and a response to DNA damage, which can cause inhibition of apoptosis of genetically altered cells [29].

Looking for ways to influence the apoptosis processes, researchers undoubtedly pay attention to the proteins of the Bcl-2 family, which are one of its main regulators.

Khodapasand E. et al. [30] demonstrated the potential prognostic value of determining Bax and Bcl-2 gene expression and establishing their ratio (Bax/Bcl-2) in colorectal cancer. Interestingly, no significant correlation was found between the expression of Bax and Bcl-2 and clinicopathological parameters of colorectal cancer. However, the Bax/Bcl-2 ratio was significantly correlated with patient age and tumor location. A decrease in the Bax/Bcl-2 ratio was found in patients over 50 years of age. Also, the Bax/Bcl-2 ratio was significantly lower in resected colon tumors compared with sigmoid, rectosigmoid, and rectal tumors. This suggests a correlation between age and tumor location and the ratio of Bax/Bcl-2 expression. This allows us to talk about the prognostic value of such an indicator as a potential molecular marker of colorectal cancer.

The human genome contains a large number of genetic variations, such as insertions/deletions of one or more nucleotides, copy number variations (CNVs) and single nucleotide polymorphisms (SNPs), which are single nucleotide substitutions along the DNA.

SNPs are the most common formula of genetic variation. It is estimated that the human genome contains more than 10 million SNPs [31]. Huang M.Y. et al. [32] revealed the presence of polymorphic changes in a number of genes regulating the activity of various enzymes in patients with colorectal cancer.

They also found that genotype distributions have ethnic variations.

A number of studies have reported the cyclooxygenase 2 (COX-2) rs689466 polymorphism to be a susceptibility locus for colorectal cancer (CRC), but their findings have been inconsistent. Zhang Y.C. et al. [33] performed a meta-analysis to determine the effect of this polymorphism more precisely on the risk of CRC. The authors conducted a detailed analysis of more than 160 scientific articles and concluded that the COX-2 rs689466 polymorphism is not correlated with colorectal cancer risk in all populations. To a greater extent, this applies to Caucasians. Nevertheless, according to the authors of this study, this conclusion needs to be confirmed by further studies in other ethnic groups.

Several studies have demonstrated an association between DNA repair gene polymorphisms and increased risk of CRC in different populations, as well as treatment outcomes. Improving the current understanding of the impact of DNA repair gene polymorphisms on CRC risk and treatment may have a diagnostic and prognostic role in CRC patients [34].

Bcl-2 is critical for carcinogenesis. Despite this, the number of studies devoted to establishing the role of Bcl-2 SNPs is limited, and some studies on the association of Bcl-2 promoter polymorphisms with susceptibility to various types of cancer are somewhat contradictory.

According to a meta-analysis on the relationship between Bcl-2 promoter SNPs and cancer susceptibility and subsequent prognosis [35], only 32 original publications were identified up to August 2016 covering two Bcl-2 promoter SNPs (rs2279115 and rs1801018).

The results of this meta-analysis showed a statistically significant association between rs2279115 and cancer susceptibility and prognosis in all four genetic models, but not in rs1801018. Subgroup analysis showed that rs2279115 was associated with a significantly higher risk of cancer in Asians but not in Caucasians. It was concluded that rs2279115 may be a tumor marker for cancer treatment in Asia.

It should be noted that to date, the number of studies on the role of SNPs in the Bcl-2 gene in tumorigenesis has increased very slightly.

We were among the first to start these studies in Ukraine.

5. Conclusions

Does Bcl-2 gene polymorphism really influence the susceptibility of patients to colorectal cancer? Will determination of Bcl-2 gene polymorphism become a routine research method in our clinics? Will such a study help form risk groups for the development of colorectal cancer? Will the study of polymorphism variants of the

Bcl-2 gene (C938A) be used for early diagnosis of colorectal cancer?

Our study showed that the CA-heterozygous variant of the polymorphic variant C938A of the Bcl-2 gene is statistically significantly associated with colon cancer in both men and women. It can be assumed that the determination of the Bcl-2 C938A gene polymorphism can be used as a molecular genetic marker to assess the risk of developing a tumor process in the colon in patients with a complicated medical history (hemorrhoids, polyps, etc.), and may also be a promising criterion for early diagnosis of colorectal cancer.

However, our current study is just the first of this kind and for proof-of-hypothesis further research is needed to clarify these issues.

References

1. Fingas CD, Katsounas A, Kahraman A, et al. Prognostic assessment of three single-nucleotide polymorphisms (GNB3 825C>T, BCL2-938C>A, MCL1-386C>G) in extrahepatic cholangiocarcinoma. Cancer Invest. 2010;28(5):472-478. doi:10.3109/07357900903095714.
2. Zinkel S, Gross A, Yang E. BCL2 family in DNA damage and cell cycle control. Cell Death Differ. 2006;13(8):1351-1359. doi:10.1038/sj.cdd.4401987.
3. Yang QF, Sakurai T, Yoshimura G, et al. Expression of Bcl-2 but not Bax or p53 correlates with in vitro resistance to a series of anticancer drugs in breast carcinoma. Breast Cancer Res Treat. 2000;61(3):211-216. doi:10.1023/a:1006474307180/
4. Zhang N, Li X, Tao K. et al. BCL-2 (-938C > A) polymorphism is associated with breast cancer susceptibility. BMC Med Genet. 2011;12:48. https://doi.org/10.1186/1471-2350-12-48https://doi.org/10.1186/1471-2350-12-48
5. Bhatt D, Verma AK, Bharti PS, Goyal Y, Alsahli MA, Almatroudi A, Rahmani AH, Almatroodi S, Joshi PC, Alam MM, Ahmad I, Zaman GS, Dev K. BCL-2 (-938C>A), BAX (-248G>A), and HER2 Ile655Val Polymorphisms and Breast Cancer Risk in Indian Population. J Oncol. 2021 Feb 25;(2021):8-8. doi: 10.1155/2021/8865624. PMID: 33708254; PMCID: PMC7932784.
6. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M. et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Nat Acad Sci USA. 2005;102:13944–13949
7. Sánchez-Beato M, Sánchez-Aguilera A, Piris MA. Cell cycle deregulation in B-cell lymphomas. Blood. 2003 Feb 15;101(4):1220-35. doi: 10.1182/blood-2002-07-2009. Epub 2002 Sep 12. PMID: 12393483.
8. Peng Y. et al. Polymorphisms of BCL2 and BAX Genes Associate with Outcomes in Advanced Non-small cell lung cancer Patients treated with platinum-based Chemotherapy. Sci. Rep. 2015;5:17766; doi: 10.1038/srep17766 (2015). PMID: 26656462; PMCID: PMC4674711.
9. Ruvolo PP, Deng X, May WS. Phosphorylation of Bcl2 and regulation of apoptosis. Leukemia. 2001 Apr;15(4):515-22. doi: 10.1038/sj.leu.2402090. PMID: 11368354.
10. Lan YJ, Yeh PS, Kao TY. et al. Anti-apoptotic BCL-2 regulation by changes in dynamics of its long unstructured loop. Commun Biol. 2020;3:668.

https://doi.org/10.1038/s42003-020-01390-6

11. Slomp A and Peperzak V. Role and Regulation of Pro-survival BCL-2 Proteins in Multiple Myeloma. Front. Oncol. 2018;8:533. doi: 10.3389/fonc.2018.00533
12. Kale, J., Osterlund, E. & Andrews, D. BCL-2 family proteins: changing partners in the dance towards death. Cell Death Differ. 2018;25:65–80. https://doi.org/10.1038/cdd.2017.186
13. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007 Feb 26;26(9):1324-37. doi: 10.1038/sj.onc.1210220. PMID: 17322918; PMCID: PMC2930981.

14. Reed JC. Bcl-2-family proteins and hematologic malignancies: history and future prospects. Blood. 2008 Apr 1;111(7):3322-30. doi: 10.1182/blood-2007-09-078162. Erratum in: Blood. 2008 Jul 15;112(2):452. PMID: 18362212; PMCID: PMC2275002.
15. Delbridge AR, Grabow S, Strasser A, Vaux DL. Thirty years of BCL-2: translating cell death discoveries into novel cancer therapies. Nat Rev Cancer. 2016; 16:99–109. doi: 10.1038/nrc.2015.17 PubMed Abstract | CrossRef Full Text | Google Scholar
16. Renault TT, Dejean LM, Manon S. A brewing understanding of the regulation of Bax function by Bcl-xL and Bcl-2. Mech Ageing Dev. 2017 Jan;161(Pt B):201-210. doi: 10.1016/j.mad.2016.04.007. Epub 2016 Apr 23. PMID: 27112371.].
17. Schenk R L, Strasser A & Dewson G. BCL-2: long and winding path from discovery to therapeutic target. Biochem. Biophys. Res. Commun. 2017;482:459–469. https://doi.org/10.1016/j.bbrc.2016.10.100. https://www.sciencedirect.com/science/article/pii/S0006291X16317867
18. Cory S, Roberts A W, Colman PM & Adams JM. Targeting BCL-2-like proteins to kill cancer cells. Trends Cancer. 2016;2:443–460. https://doi.org/10.1016/j.trecan.2016.07.001.
https://www.sciencedirect.com/science/article/pii/S2405803316300802
19. Adams J, Cory S. The BCL-2 arbiters of apoptosis and their growing role as cancer targets. Cell Death Differ. 2018;25:27–36. https://doi.org/10.1038/cdd.2017.161
20. Basu A, DuBois G, Haldar S. Posttranslational modifications of Bcl2 family members--a potential therapeutic target for human malignancy. Front Biosci. 2006 May 1;11:1508-21. doi: 10.2741/1900. PMID: 16368533
21. Nuckel H. et al. (2007) Association of a novel regulatory polymorphism (-938C>A) in the BCL2 gene promoter with disease progression and survival in chronic lymphocytic leukemia. Blood. 2007;109:290–297. doi: 10.1182/blood-2006-03-007567
22. Bachmann HS et al. (2011). Regulatory BCL2 promoter polymorphism (-938C>A) is associated with adverse outcome in patients with prostate carcinoma. International journal of cancer. Journal international du cancer. 2011;129:2390–2399.
doi: 10.1002/ijc.25904
23. Hirata H et al. (2009). The bcl2 -938CC genotype has poor prognosis and lower survival in renal cancer. The Journal of urology. 2009;182:721–727.
doi: 10.1016/j.juro.2009.03.081
24. Bachmann H S et al. (2007). The AA genotype of the regulatory BCL2 promoter polymorphism (938C>A) is associated with a favorable outcome in lymph node negative invasive breast cancer patients. Clinical cancer research: an official journal of the American Association for Cancer Research. 2007;13:5790–5797.
doi: 10.1158/1078-0432.CCR-06-2673
25. Chen K et al. (2007). Single-nucleotide polymorphisms at the TP53-binding or responsive promoter regions of BAX and BCL2 genes and risk of squamous cell carcinoma of the head and neck. Carcinogenesis. 2007;28:2008–2012.
doi: 10.1093/carcin/bgm172
26. Hirata H et al. (2009). Bcl2 -938C/A polymorphism carries increased risk of biochemical recurrence after radical prostatectomy. The Journal of urology. 2009;181: 1907–1912. doi: 10.1016/j.juro.2008.11.093
27. Li W, Qian C, Wang L, Teng H & Zhang L. Association of BCL2-938C>A genetic polymorphism with glioma risk in Chinese Han population. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35:2259–2264. doi: 10.1007/s13277-013-1299-5.
28. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statis-tics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. doi:10.3322/caac.21492
29. Rytsyk O, Soroka Y, Shepet I, et al. Experimental Evaluation of the Effectiveness of Resveratrol as an Antioxidant in Colon Cancer Prevention. Natural Product Communications. June 2020. doi:10.1177/1934578X20932742
30. Khodapasand E, Jafarzadeh N, Farrokhi F, Kamalidehghan B, Houshmand M. Is Bax/Bcl-2 ratio considered as a prognostic marker with age and tumor location in colorectal cancer? Iran Biomed J. 2015;19(2):69-75. doi: 10.6091/ibj.1366.2015. PMID: 25864810; PMCID: PMC4412916.
31. Sameer AS. Colorectal cancer: molecular mutations and polymorphisms. Front Oncol. 2013 May 13;3:114. doi: 10.3389/fonc.2013.00114. PMID: 23717813; PMCID: PMC3651991.
32. Huang MY, Fang WY, Lee SC et al. ERCC22251A>C genetic polymorphism was highly correlated with early relapse in high-risk stage II and stage III colorectal cancer patients: A preliminary study. BMC Cancer. 2008;8:50. https://doi.org/10.1186/1471-2407-8-50
33. Zhang YC, Zhao H, Chen C et al. COX-2 gene rs689466 polymorphism is associated with increased risk of colorectal cancer among Caucasians: a meta-analysis. World J Surg Onc 18, 192 (2020). https://doi.org/10.1186/s12957-020-01957-x
34. Al-Shaheri FN, Al-Shami KM, Gamal EH, Mahasneh AA, and Ayoub NM, Association of DNA repair gene polymorphisms with colorectal cancer risk and treatment outcomes, Exp. Mol. Pathol. 2020;(113):104364.
35. Yao Z, Yang B, Liu Z, Li W, He Q, Peng X. Genetic polymorphisms of Bcl-2 promoter in cancer susceptibility and prognosis: a meta-analysis. Oncotarget. 2017;8: 25270-25278. https://www.oncotarget.com/article/15751/text/
36. Huang B, Liu X, Sun C, Wang L, Yang L. Association of single nucleotide polymorphisms in the coding region of Bcl-2 with the occurrence and prognosis of colorectal cancer: A case-control study. Cancer Biomark. 2017;18(4):433-439. doi: 10.3233/CBM-160378. PMID: 28106544.