# The effect of exercise-induced dopamine replica in colon cancer cell line (HT-29) prevention via apoptotic signaling pathway

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**Running Title:** Analysis of protective role of exercise induced dopamine replica in colon cancer cell line (HT-29) prevention through apoptotic signaling mechanism.

**Type of article:** Original Research

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**ABSTRACT:**

**BACKGROUND:**
During exercise we release a lot of hormones and neurotransmitters. Dopamine is one among the hormones which is released in several parts of the mid brain during exercise. It is said that exercise can help to avoid many diseases and even cancer.. In 2020 the number of cases diagnosed were 104,610 all around the world .In this study we analysed the role of endogenous dopamine replica over gene expression of Apoptotic and tumor suppressor gene against colon cancer prevention.

**MATERIALS AND METHODS**:

Human colon cancer cell line (HT -29) was brought from NCCS, Pune, India. Cell viability test and Gene expression analysis were carried out for apoptotic and tumor suppressor gene using MTT and PCR respectively. The results were analyzed using appropriate statistical tools using ANOVA and Duncan's test.

**RESULTS** :
Bcl-2(B-cell lymphoma 2) RNA gene expression was reduced on induction in dosage of dopamine in a significant variation in comparison to control as Bcl-2 which is an anti-apoptotic gene. The second gene, Bcl-xl RNA gene expression was reduced on induction in dosage of dopamine in a significant variation in comparison to control as Bcl-xl is an anti-apoptotic gene. P53(tumour protein 53) RNA gene expression was increased on induction in dosage of dopamine in a significant difference in comparison to control as P53 is a tumour suppressor gene.

**CONCLUSION**:
In the present it was observed that the selected cell variables Bcl-2, Bcl-xl and P53 showed significant results on induction of dopamine in colon cancer cells (HT-29) thus we conclude that dopamine may have anti-apoptotic effects in colon cancer prevention. Since this study shows a basic idea on what dopamine can do in these genes,

**KEYWORDS**: exercise; colon cancer; HT-29 CELLS; Bcl-2; Bcl-xl; P53; Innovative technique

**INTRODUCTION**
Health is not only about keeping us away from disease but it's about the well-being of one's Physical, mental, and social well-being. By keeping yourself in a physiologic state of well-being which allows us to meet the demands of daily living. Moreover, exercising has attracted increased interest in rehabilitation of not only oncological patients but also the lives of normal individuals during and after the year 2020. During exercise we release a lot of hormones such as Endorphins, these hormones that interact with the receptors in your brain to reduce endorphins that are released. Endorphins also trigger a happy feeling within your body

that is accompanied by a more positive outlook on your day today. One such endorphins that are released is dopamine.

The hypothalamus releases dopamine, which is one of the endorphins. Its activity as a hormone inhibits the production of prolactin from the pituitary's anterior lobe. It has an effect on the sympathetic nervous system (1). Dopamine induction causes a rise in heart rate and blood pressure.. Dopamine is also important in the treatment of brain diseases (2). Parkinson's disease and dopa-responsive dystonia are the results. Cancer has invaded the lives of many individuals in the world and millions of cases were recorded in the past decade. Cancer occurs when the cells divide uncontrollably due to mutations caused by external factors. Cancer is a type of aberrant cell growth that tends to spread uncontrollably and, in some situations, metastasize. [3]. The previous research experience [4] [5,6] [5789108,101112] [13] have led us to concentrate on the study.

Everyone can get cancer depending on their lifestyle or by any case of family history of cancer. Any imbalance in the function of DNA repair genes, oncogenes, tumour suppressor gene and apoptotic gene will lead to cancer. Colon carcinoma is the second most common cause of death from cancer[14]. Any mutations in MSH2,MSH6 both on chromosome 2 and MLH1 on chromosome 3 will lead to colon cancer[15]. Studies at molecular levels were performed by our team of researches which insisted us to proceed this study [16–23],[24],[25],[26],[27,28],[29],[30],[31–35] The aim of the study is to analyse the protective role in exercise induced dopamine in colon cancer prevention.

## Materials and Methods

### Procedure

Cell line centre, Pune, India, provided the human colon cancer cell line HT29. Tissues were cultured in RPMI media with 10% foetal bovine serum, 100 U/ml penicillin, and 100 g/ml streptomycin at 37 degrees Celsius and 5% CO2.The MTT test was used to measure cell growth. (HT-29) tissues were sown in 96-well plates with 5x104/200l and grown overnight. Six duplicate wells were used in each treatment. All of the tissues were then grown for another 48 hours. The experiment was carried out three times. The MTT absorbance in negative control tissues was employed as a 0 percent cell inhibition measurement. The expression status of m RNA was analysed by Polymerase chain reaction for identifying the fold change of BCL2, BCL-XL and P53 m RNA expression over control samples. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to determine the significance of the results, with significance set at 0.05.

## RESULT:

Dopamine was found to reduce the abnormal proliferation of cells. (Figure 1) (Table 1).
Effect of dopamine on Bcl-2 mRNA expression on the HT-29 cancer cells found to have reduction in m RNA expression of Bcl2 in different doses of dopamine. (Figure 2). There was

significant reduction in expression of Bcl-xl in comparison to untreated group. (Figure 3). The P53 expression was found to be increased significantly on administration of dopamine. (Figure 4)

**Table1: Assessment of Cell Viability**

| Dopamine Concentration (µM) | Cell viability (%) |
|---|---|
| 0 | 100 |
| 100 | 78 |
| 200 | 50 |
| 400 | 40 |

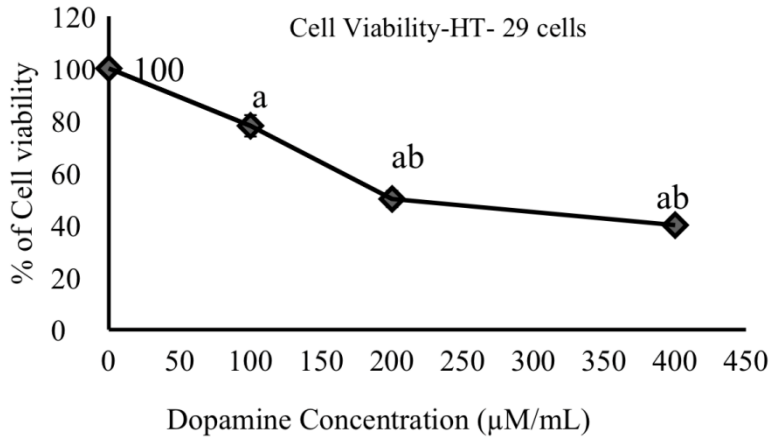


**Fig 1: Effect of dopamine on cell viability in HT-29 cells.**

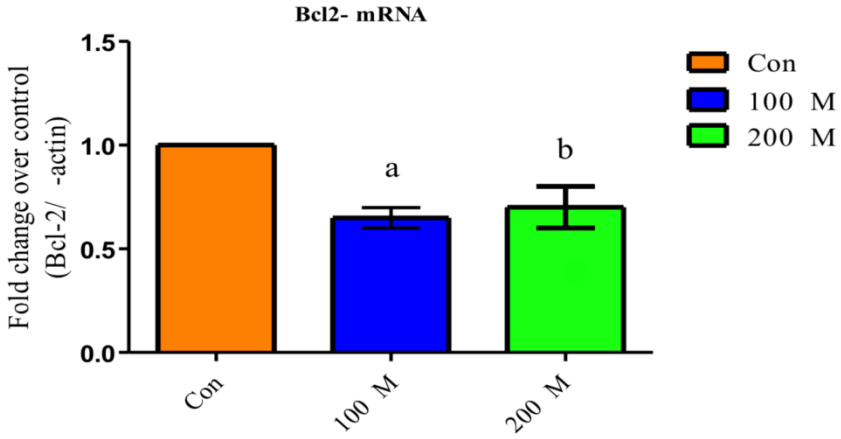


**Fig 2 : Effect of dopamine on Bcl-2 mRNA expression in HT-29 cells.**

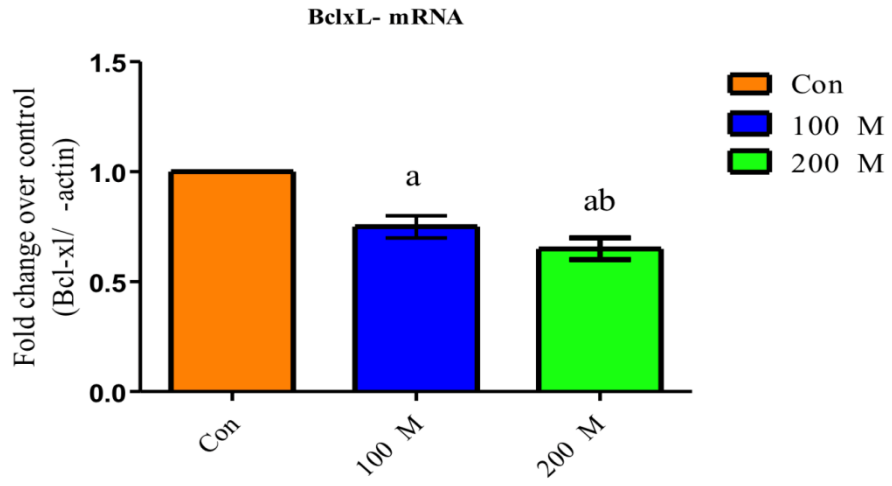


**Fig 3: Effect of dopamine on Bcl-xl mRNA expression in HT-29 cells.**

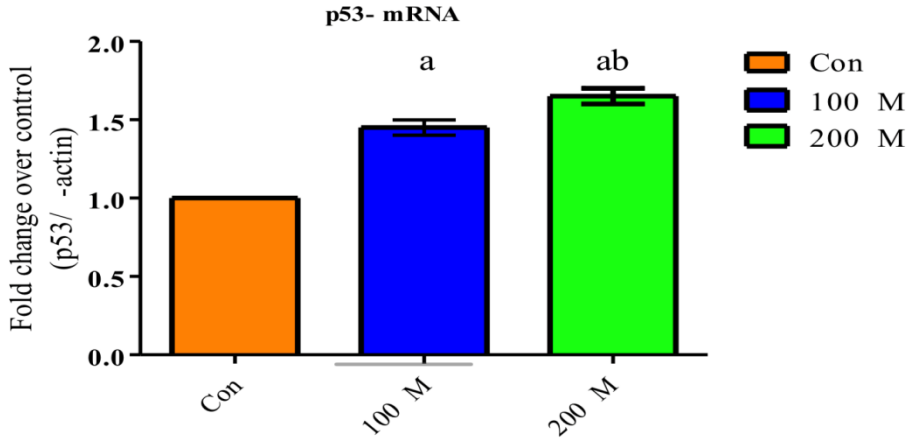


**Fig 4: Effect of dopamine on p53mRNA expression in HT-29 cells.**

## DISCUSSION

The effect of dopamine on cell viability in HT-29 cells was shown to be significantly reduced when the concentration of dopamine was increased in this investigation. A similar trend was found with Bcl-xl gene expression, which decreased significantly as dopamine concentration increased.As P53 is a tumor suppressor gene it showed a significant increase in induction of dopamine. The selected cell variables of dopamine showed positive effects in colon cancer prevention.

v.[36].This following positive result for the mRNA expression of Bcl-2 is supported In a recent article made by distelhorst in the year 2019 stated various new therapeutic methods to treat cancer.[37]. Apart from the secretions of hormones in our body, In a recent research made a study on mRNA expression of Bcl-2 on colon cancer used the alkaloid[38]. Bcl-xl was considered one of the antiapoptotic protein that was overactivated in colon cancer. in a study supporting the results of antiapoptotic property expressed in this study also concluded that targeting of BCL-XL is efficient and was safe when trialed on preclinical CRC

models[39].In the present study it was evident that if Bcl-xl had expressed its anti apoptotic property on HT-29 cells but in a study made by trisciuoglio in 2017 stated that on overexpression of Bcl -xl may lead to progression in tumor growth[40].

.In our present study it showed a positive result in the mRNA gene expression of P53 as it is a tumor suppressor gene when exposed to dopamine it increases in expression hence it helps to inhibit colon cancer. In a study p53 was studied , whether it had anticancer effects on breast cancer, they concluded , they found that it had established roles in stimulating cell-cycle arrest and apoptosis and also control of metabolic function[41]. An integral analysis was made in order to find whether there is a better prognosis for patients with colon cancer and it was concluded that in the absence of p53 or tp53 patients did not show better prognosis in colon cancer[42]. In a study it showed that Patients with mutant *p53* genes are often resistant to current therapies, conferring poor prognosis in colon cancer.[43]. Limitations of this study included less sample size hence we cannot conclude and large scale research on this study can be conducted to make the results more evident.

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**CONCLUSION**:

The study concludes that there is a protective role of exercise induced dopamine in colon cancer prevention through apoptotic signaling mechanism since gene expression of Bcl-2 and Bcl-xl is reduced since they both had anti-apoptotic gene function and p53 expression is increased since it is a tumour suppressor gene hence we conclude that dopamine may have a protective role in colon cancer prevention.

ACKNOWLEDGMENT: We thank saveetha dental college and hospitals for the successful completion of the study.

SOURCE OF FUNDING:

The present study was supported by the following agencies.

- Saveetha Institute of Medical and Technical Sciences (SIMATS)
- Saveetha Dental College and HospitalsDental College and Hospitals
- Saveetha University
- NRV Hospital Chennai

CONFLICT OF INTEREST: All the authors declare that there was no conflict of interest in the present study.

**REFERENCE**

1. Bogacz R. Dopamine role in learning and action inference [Internet]. Vol. 9, eLife. 2020. Available from: http://dx.doi.org/10.7554/elife.53262

2. Kiyatkin EA, Rebec GV. Dopamine-independent action of cocaine on striatal and accumbal neurons [Internet]. Vol. 12, European Journal of Neuroscience. 2000. p. 1789–

800. Available from: http://dx.doi.org/10.1046/j.1460-9568.2000.00066.x

3. Higley C, Hsu A, Park BU, Pang M. Back to Basics: History and Physical Examination Uncover Colonic Metastasis in a Patient With Remote History of Breast Cancer. ACG Case Rep J. 2020 Dec;7(12):e00494.

4. Shruthi M, Preetha S. Effect of Simple Tongue Exercises in Habitual Snorers [Internet]. Vol. 11, Research Journal of Pharmacy and Technology. 2018. p. 3614. Available from: http://dx.doi.org/10.5958/0974-360x.2018.00665.0

5. Preetha S, Packyanathan J. Comparison of the effect of Yoga, Zumba and Aerobics in controlling blood pressure in the Indian population [Internet]. Vol. 9, Journal of Family Medicine and Primary Care. 2020. p. 547. Available from: http://dx.doi.org/10.4103/jfmpc.jfmpc_607_19

6. J SK, Saveetha Dental College and Hospitals, Road PH, Chennai, Tamilnadu, Preetha S, et al. Effect of aerobics exercise and yoga on blood pressure in hypertensives [Internet]. Vol. 6, International Journal of Current Advanced Research. 2017. p. 3124–6. Available from: http://dx.doi.org/10.24327/ijcar.2017.3126.0200

7. Prathap L, Suganthirababu P, Ganesan D. Fluctuating Asymmetry of Dermatoglyphics and DNA Polymorphism in Breast Cancer Population [Internet]. Vol. 10, Indian Journal of Public Health Research & Development. 2019. p. 3574. Available from: http://dx.doi.org/10.5958/0976-5506.2019.04141.x

8. Lavanya J, Prathap S, Alagesan J. Digital and palmar dermal ridge patterns in population with breast carcinoma. Biomedicine. 2014 Jul 1;34(3):315–21.

9. Prathap L, Jagadeesan V. Association of quantitative and qualitative dermatoglyphic variable and DNA polymorphism in female breast cancer population. Online J Health [Internet]. 2017; Available from: https://www.researchgate.net/profile/Prathap_Suganthirababu/publication/321606278_Association_of_Quantitative_and_Qualitative_Dermatoglyphic_Variable_and_DNA_Polymorphism_in_Female_Breast_Cancer_Population/links/5a28c8f1a6fdcc8e8671c0cd/Association-of-Quantitative-and-Qualitative-Dermatoglyphic-Variable-and-DNA-Polymorphism-in-Female-Breast-Cancer-Population.pdf

10. Lavanya J, Kumar VJ, Sudhakar N, Prathap S. Analysis of DNA repair genetic polymorphism in breast cancer population. Int J Pharma Bio Sci [Internet]. 2015; Available from: https://scholar.google.ca/scholar?cluster=8949053652564257518&hl=en&as_sdt=0,5&sciodt=0,5

11. Prathap L, Suganthirababu P. Estrogen Exposure and its Influence in DNA Repair Genetic Variants in Breast Cancer Population [Internet]. Vol. 13, Biomedical and Pharmacology Journal. 2020. p. 1321–7. Available from:

http://dx.doi.org/10.13005/bpj/2001

12. Ravikumar H, Prathap L, Preetha S. ANALYSIS OF PALMAR ATD ANGLE IN POPULATION WITH MALOCCLUSION. 2020 Jan 1;1174–82.

13. Prathap L. INTERPLAY OF OXIDATIVE STRESS AND LIPOPROTEINS IN BREAST CARCINOMA INITIATION, PROMOTION AND PROGRESSION -A SYSTEMATIC REVIEW. PalArch's Journal of Archaeology of Egypt/ Egyptology [Internet]. 2021 Jan 7 [cited 2021 Mar 9];17(7). Available from: http://dx.doi.org/

14. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, et al. Identification and expansion of human colon-cancer-initiating cells. Nature. 2006 Nov 19;445(7123):111–5.

15. Luo R, Guo W, Wang H. A comprehensive analysis of tumor microenvironment-related genes in colon cancer. Clin Transl Oncol [Internet]. 2021 Mar 10; Available from: http://dx.doi.org/10.1007/s12094-021-02578-w

16. Sekar D, Lakshmanan G, Mani P, Biruntha M. Methylation-dependent circulating microRNA 510 in preeclampsia patients. Hypertens Res. 2019 Oct;42(10):1647–8.

17. Princeton B, Santhakumar P, Prathap L. Awareness on Preventive Measures taken by Health Care Professionals Attending COVID-19 Patients among Dental Students. Eur J Dent. 2020 Dec;14(S 01):S105–9.

18. Logeshwari R, Rama Parvathy L. Generating logistic chaotic sequence using geometric pattern to decompose and recombine the pixel values. Multimed Tools Appl. 2020 Aug;79(31-32):22375–88.

19. Johnson J, Lakshmanan G, M B, R M V, Kalimuthu K, Sekar D. Computational identification of MiRNA-7110 from pulmonary arterial hypertension (PAH) ESTs: a new microRNA that links diabetes and PAH. Hypertens Res. 2020 Apr;43(4):360–2.

20. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res. 2020 Jul;43(7):729–30.

21. Pujari GRS, Subramanian V, Rao SR. Effects of Celastrus paniculatus Willd. and Sida cordifolia Linn. in Kainic Acid Induced Hippocampus Damage in Rats. Ind J Pharm Educ. 2019 Jul 3;53(3):537–44.

22. Rajkumar KV, Lakshmanan G, Sekar D. Identification of miR-802-5p and its involvement in type 2 diabetes mellitus. World J Diabetes. 2020 Dec 15;11(12):567–71.

23. Ravisankar R, Jayaprakash P, Eswaran P, Mohanraj K, Vinitha G, Pichumani M. Synthesis, growth, optical and third-order nonlinear optical properties of glycine sodium nitrate single crystal for photonic device applications. J Mater Sci: Mater Electron. 2020 Oct;31(20):17320–31.

24. Wu S, Rajeshkumar S, Madasamy M, Mahendran V. Green synthesis of copper nanoparticles using Cissus vitiginea and its antioxidant and antibacterial activity against urinary tract infection pathogens. Artif Cells Nanomed Biotechnol. 2020 Dec;48(1):1153–8.

25. Vikneshan M, Saravanakumar R, Mangaiyarkarasi R, Rajeshkumar S, Samuel SR, Suganya M, et al. Algal biomass as a source for novel oral nano-antimicrobial agent. Saudi J Biol Sci. 2020 Dec;27(12):3753–8.

26. Alharbi KS, Fuloria NK, Fuloria S, Rahman SB, Al-Malki WH, Javed Shaikh MA, et al. Nuclear factor-kappa B and its role in inflammatory lung disease. Chem Biol Interact. 2021 Aug 25;345:109568.

27. Rao SK, Kalai Priya A, Manjunath Kamath S, Karthick P, Renganathan B, Anuraj S, et al. Unequivocal evidence of enhanced room temperature sensing properties of clad modified Nd doped mullite Bi2Fe4O9 in fiber optic gas sensor [Internet]. Vol. 838, Journal of Alloys and Compounds. 2020. p. 155603. Available from: http://dx.doi.org/10.1016/j.jallcom.2020.155603

28. Bhavikatti SK, Karobari MI, Zainuddin SLA, Marya A, Nadaf SJ, Sawant VJ, et al. Investigating the Antioxidant and Cytocompatibility of Mimusops elengi Linn Extract over Human Gingival Fibroblast Cells. Int J Environ Res Public Health [Internet]. 2021 Jul 4;18(13). Available from: http://dx.doi.org/10.3390/ijerph18137162

29. Marya A, Karobari MI, Selvaraj S, Adil AH, Assiry AA, Rabaan AA, et al. Risk Perception of SARS-CoV-2 Infection and Implementation of Various Protective Measures by Dentists Across Various Countries. Int J Environ Res Public Health [Internet]. 2021 May 29;18(11). Available from: http://dx.doi.org/10.3390/ijerph18115848

30. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021 Jun;126:105132.

31. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019 Dec;90(12):1441–8.

32. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species [Internet]. Vol. 94, Archives of Oral Biology. 2018. p. 93–8. Available from: http://dx.doi.org/10.1016/j.archoralbio.2018.07.001

33. Uma Maheswari TN, Nivedhitha MS, Ramani P. Expression profile of salivary micro RNA-21 and 31 in oral potentially malignant disorders. Braz Oral Res. 2020 Feb 10;34:e002.

34. Gudipaneni RK, Alam MK, Patil SR, Karobari MI. Measurement of the Maximum Occlusal Bite Force and its Relation to the Caries Spectrum of First Permanent Molars in Early Permanent Dentition. J Clin Pediatr Dent. 2020 Dec 1;44(6):423–8.

35. Chaturvedula BB, Muthukrishnan A, Bhuvaraghan A, Sandler J, Thiruvenkatachari B. Dens invaginatus: a review and orthodontic implications. Br Dent J. 2021 Mar;230(6):345–50.

36. Sarkar C, Chakroborty D, Chowdhury UR, Dasgupta PS, Basu S. Dopamine increases the efficacy of anticancer drugs in breast and colon cancer preclinical models. Clin Cancer Res. 2008 Apr 15;14(8):2502–10.

37. Distelhorst CW, Bootman MD. Creating a New Cancer Therapeutic Agent by Targeting the Interaction between Bcl-2 and IP3Receptors [Internet]. Vol. 11, Cold Spring Harbor Perspectives in Biology. 2019. p. a035196. Available from: http://dx.doi.org/10.1101/cshperspect.a035196

38. Lee JS, Jung W-K, Jeong MH, Yoon TR, Kim HK. Sanguinarine Induces Apoptosis of HT-29 Human Colon Cancer Cells via the Regulation of Bax/Bcl-2 Ratio and Caspase-9-Dependent Pathway [Internet]. Vol. 31, International Journal of Toxicology. 2012. p. 70–7. Available from: http://dx.doi.org/10.1177/1091581811423845

39. Scherr A-L, Mock A, Gdynia G, Schmitt N, Heilig CE, Korell F, et al. Identification of BCL-XL as highly active survival factor and promising therapeutic target in colorectal cancer. Cell Death Dis. 2020 Oct 17;11(10):875.

40. Trisciuoglio D, Tupone MG, Desideri M, Di Martile M, Gabellini C, Buglioni S, et al. BCL-XL overexpression promotes tumor progression-associated properties [Internet]. Vol. 8, Cell Death & Disease. 2017. Available from: http://dx.doi.org/10.1038/s41419-017-0055-y

41. Wang X, Simpson ER, Brown KA. p53: Protection against Tumor Growth beyond Effects on Cell Cycle and Apoptosis [Internet]. Vol. 75, Cancer Research. 2015. p. 5001–7. Available from: http://dx.doi.org/10.1158/0008-5472.can-15-0563

42. Sarasqueta AF, Forte GI, Corver WE, de Miranda NF, Ruano D, van Eijk R, et al. Integral analysis of p53 and its value as prognostic factor in sporadic colon cancer [Internet]. Vol. 13, BMC Cancer. 2013. Available from: http://dx.doi.org/10.1186/1471-2407-13-277

43. Zhou L, Feng Y, Jin Y, Liu X, Sui H, Chai N, et al. Abstract 1762: Verbascoside promotes apoptosis by regulating HIPK2-p53 signaling in human colorectal cancer [Internet]. Experimental and Molecular Therapeutics. 2015. Available from: http://dx.doi.org/10.1158/1538-7445.am2015-1762