miR-485-3p suppresses colorectal cancer via targeting TPX2

Taherdangkoo K1, Kazemi Nezhad SR2, Hajjari MR1, Tahmasebi Birgani M4

Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
kh.taherdangkoo@gmail.com

ABSTRACT
BACKGROUND: Colorectal cancer (CRC), is the third most common cancer type. MicroRNAs and their roles in cancer progression have gained considerable attention in the scientific community. miR-485-3p has been identified to be abnormally expressed in different types of cancer, but its expression level, biological function, and underlying pathways are still unclear in CRC. Targeting Protein for Xenopus Kinesin-like Protein 2 (TPX2) is a nuclear protein which plays vital roles in cancer progression and mitotic spindle assembly. TPX2 is overexpressed in various malignancies and has been predicted as an indirect target of miR-485-3p. This study aims to investigate the miR-485-3p and TPX2 expression level, their potential correlation, and underlying molecules like P53 and P21 in forty-one pairs of colorectal cancer tissues compared to matched non-cancerous ones.

MATERIALS AND METHODS: We used forty-one pairs of CRC fresh tissue samples and their adjacent normal ones for RNA extraction. After cDNA synthesis, the expression level of miR-485-3p, TPX2, P53 and P21 were determined by Real-time PCR.

RESULTS AND CONCLUSIONS: The results revealed that miR-485-3p was significantly downregulated and TPX2 was highly upregulated in CRC tissues. Moreover, miR-485-3p was negatively correlated with TPX2 expression and positively correlated with P21 expression. We present miR-485-3p as a suppressor for colorectal cancer (Tab. 2, Fig. 8, Ref. 44). Text in PDF www.els.sk.

KEY WORDS: colorectal cancer, microRNA, miR-485-3p, TPX2.

Introduction
Colorectal cancer (CRC) is the third most prevalent cancer and the second cause of cancer-related deaths around the world (1). It is a common malignancy in patients between the ages of 65 and 74, and the incidence rate is increasing rapidly especially in low-income countries (2). Several risk factors are involved in CRC carcinogenesis, for instance: increased age, male sex, inheritance, colon inflammatory disease, the presence of polyps, intake large of amounts of meat and processed food, smoking, obesity, sedentary life and so on (1, 3–6). Currently, despite excessive advances throughout the years, the fundamental helpful strategy for treating CRC patients is still surgery pursued by chemotherapy and radiotherapy depending on patient state, although the survival rate is still low (7). Since colorectal cancer has a long preclinical-stage and there is not any obvious early symptom for this malignancy, the better understanding of unclear molecular CRC carcinogenesis pathways will be helpful for finding putative biomarkers, which can be used for diagnosis and prognosis of patients with this disease.

microRNAs (miRNAs) are small, single-stranded and evolutionary highly conserved non-coding RNAs with 19 to 25 nucleotides length (8–10). miRNAs can negatively regulate expression of their target mRNAs at the post-transcriptional level by partially specific binding to their 3′ untranslated region (3′-UTR) which leads to mRNA degradation or translational inhibition. Furthermore, miRNAs play crucial roles in physiological and pathological processes such as proliferation, differentiation, apoptosis, angiogenesis, inflammation, and carcinogenesis (11–14). miRNAs can act as oncogenes or tumor-suppressors in progression of different cancers (15).

miR-485-3p is located at 101055419-101055491 (+) gene region on chromosome 14q32.31. Emerging evidences demonstrate that miR-485-3p can act as an onco gene in gastric cancer (16), hepatocellular carcinoma (17, 18), prostate cancer (19, 20), and lymphoma (21). In contrast, studies showed that miR-485-3p can act as a tumor-suppressor in osteosarcoma (22), NSCLC lung cancer (23, 24), glioblastoma (25) and breast cancer (26). However, its role in initiation and progression of colorectal cancer has remained disputed. Previous researches suggested that miR-485-3p could probably regulate TPX2 at the post-transcriptional level indirectly but the correlation has not been yet investigated in any types of cancer (20, 21, 27, 28).

The Targeting Protein for Xenopus Kinesin-like Protein 2 (TPX2) is a microtubule nucleation factor which is encoded by a gene located on chromosome 20q11.2. TPX2 plays a critical role...
Taherdangkoo K et al. miR-485-3p suppresses colorectal cancer via targeting TPX2

In spindle assembly by having an interaction with Aurora-A in a RAN-GTP dependent pathway (29, 30). In addition, TPX2 is recognized as an amplification marker and acts as an oncogene. Its aberrant expression contributes to cell cycle-distraction, apoptosis, aneuploidy, polyploidy and cancer progression (31). Recent studies indicated that TPX2 is involved in different types of cancer such as pancreatic cancer (29), bladder cancer (30), cervical cancer (32), hepatocellular carcinoma (33), thyroid cancer (34), gastric cancer (35), prostate cancer (36), kidney cancer (37), and breast cancer (38). A recent research clarified that TPX2 silencing would decrease PI3K and AKT phosphorylation leading to the increase of P53 and P21 expression level (39).

In this study, we investigate miR-485-3p and TPX2 expression level in colorectal cancer tissues compared to adjacent non-cancerous ones. To the best of our knowledge, this is the first study reporting the potential tumor suppressor role of miR-485-3p in colorectal cancer and its correlation with TPX2 and its downstream genes including P21 and P53 expression level.

### Materials and methods

#### Bioinformatic analysis

We applied bioinformatic tools to detect the putative miRNA in colorectal cancer progression and its underlying molecules. We selected miR-485-3p via using miRNA Pathway Dictionary Database (miRPathDB) (https://mpd.bioinf.uni-sb.de/). For detecting probable target genes of miR-485-3p, we used various databases such as: TargetScan (www.targetscan.org), miRWalk (mirwalk.umm.uniheidelberg.de), miRTar (mirTar.mbc.nctu.edu.tw) and miRTarBase (miRTarbase.mbc.nctu.edu.tw) and available literatures (20, 21, 27, 28). Afterwards, we checked miR-485-3p expression profile in CRC tissues by using YM500v3 (http://driverdb.tms.cmu.edu.tw/ym500v3) and database of Differentially Expressed MiRNAs in human Cancers (dbDEMC2.0) (www.picb.ac.cn/dbDEMC).

#### Human tissue samples collection and preparation

Forty-one pairs of CRC fresh tissue samples and their adjacent normal ones were obtained from Iran National Tumor Bank, examined and confirmed by pathologists. All tissue samples were stored under -80°C before using for RNA extraction. The experiments were approved by the Medical Ethics Committee of Shahid Chamran University of Ahvaz.

#### RNA extraction

Total RNA was extracted from tissue samples based on acid guanidinium phenol chloroform process via using RNX-PLUS solution (CinnaGen, Tehran, Iran) according to the manufacturer’s

---

Tab. 1. The sequence of used primers.

| Name    | Primer Sequence ( 5’ to 3’ ) | PCR product length (nucleotides) |
|---------|-----------------------------|------------------------------------|
| SNORD47 | Stem-loop GTCGATCCAGTGAGGG | 63                                 |
|         | Forward ATCACCTGAAAACCCTTCA |                                   |
|         | Reverse GTCGAGGTTGCGAGGT    |                                   |
| miR-485-3p | Stem-loop GTCGATCCAGTGAGGG | 61                                 |
|         | Forward CAGTCTACACGCGTCTTC |                                   |
|         | Reverse CCAGTGCGGCTCCCTG    |                                   |
| GAPDH   | Forward GTGAAACCATTGAGAATAGA | 123                                |
|         | Reverse CATGAGTTCCTCCAGTAC  |                                   |
| TPX2    | Forward CCACCAAGAAATGAGGAGA | 101                                |
|         | Reverse TCTTTGCTCTGGGATTTGG |                                   |
| P53     | Forward TAACAGTTCTCGATGGGCC  | 121                                |
|         | Reverse AGGCAGGCAACACAGCC    |                                   |
| P21     | Forward TGGAGACTCTCAGGGTCA   | 66                                 |
|         | Reverse CGCGGTTTGGAGTGTAGAA |                                   |

---

**Fig. 1.** miR-485-3p is low expressed in CRC tissues. Real-time PCR was performed in 41 pairs of CRC fresh tissue samples and their adjacent normal ones (p < 0.0001).

**Fig. 2.** miR-485-3p downregulation graph in colorectal cancer tissues from YM500v3.
instructions. Total RNA purity and quantity were measured by Nanodrop Spectrophotometry (Thermo Fisher Scientific, USA).

**Complementary DNA (cDNA) synthesis**

Total RNA was served as the template in reverse transcription reaction to make cDNA by using PrimeScript™ RT Reagent Kit (Takara Holdings, Kyoto, Japan). We used specific stem-loop RT primers (Macrogen Inc. Seoul, South Korea) for miR-485-3p and SNORD47 cDNA synthesis according to the following protocol: 16 °C for 30 minutes, 42 °C for 30 minutes and 85 °C for 5 minutes. Oligo dT and random hexamer were used for TPX2, P53, P21 and GAPDH cDNA synthesis based on to the following protocol: 37 °C for 15 minutes and 85 °C for 5 minutes.

**Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)**

Synthesized cDNA was applied for Real-time PCR by using ABI Step One (Applied Biosystems, USA) with SYBER Green® Premix Ex Taq™ II master mix (Takara Holding, Kyoto, Japan) according to manufacturer’s instructions. The Real-time PCR reactions were performed by 40 cycles as follows: an initial hot-start for activating Taq polymerase enzyme at 95 °C for 30 seconds, denaturation at 95 °C for 5 seconds, annealing and elongation at 60 °C for 34 seconds (for miR-485-3p and SNORD47 at 62 °C for 34 seconds). The primers for SNORD47, GAPDH, and P21 were used from literature (40, 41). The primers for miR-485-3p, TPX2 and P53 are presented in Table 1. SNORD47 and GAPDH genes were used as reference internal control. Specificities of the PCR products were assessed by the sizes of the Real-time PCR products via agarose gel electrophoresis, as well as by the uniqueness of the melt curves of products.

**Data analysis**

We used 2−ΔΔct method for analyzing the Real-time PCR results. The relative expression of miR-485-3p was assessed in comparison to SNORD47 expression and the relative expression of TPX2, P53 and P21 were assessed in comparison to GAPDH expression. All statistical analyses were performed by GraphPad Prism® (GraphPad Prism software, USA). All results were expressed as the mean ± standard deviation (SD). Two-tailed paired student’s t-test was used for comparing different groups. The relationship between miR-485-3p and clinicopathological factors was analyzed by unpaired student’s t-test. Spearman correlation was used for analyzing the correlation between miR-485-3p and clinicopathological factors was analyzed by unpaired student’s t-test. Spearman correlation was used for analyzing the correlation between miR-485-3p and clinicopathological factors was analyzed by unpaired student’s t-test. Spearman correlation was used for analyzing the correlation between miR-485-3p and clinicopathological factors was analyzed by unpaired student’s t-test. Spearman correlation was used for analyzing the correlation between miR-485-3p and clinicopathological factors was analyzed by unpaired student’s t-test. Spearman correlation was used for analyzing the correlation between miR-485-3p and clinicopathological factors was analyzed by unpaired student’s t-test. Spearman correlation was used for analyzing the correlation between miR-485-3p and clinicopathological factors was analyzed by unpaired student’s t-test. Spearman correlation was used for analyzing the correlation between miR-485-3p and clinicopathological factors was analyzed by unpaired student’s t-test.

**Results**

miR-485-3p is extensively downregulated in colorectal cancer tissues

We collected 41 pairs of fresh tissue samples to evaluate the expression alteration of miR-485-3p in colorectal cancer tissues compared to adjacent non-cancerous ones. The data from RT-PCR showed that the RNA expression level of miR-485-3p is significantly lower in CRC tissues (Fig. 1) which validates the obtained results from YM500v3 (Fig. 2) and dbDEMC2.0 (logFC = −0.63). We also assessed the relationship between miR-485-3p and clinicopathological features from which no association was observed (Tab. 2).

---

**Tab. 2. Clinicopathological features and their correlation with miR-485-3p expression in 41 CRC patients.**

| Variables | Number of cases | p   |
|-----------|----------------|-----|
| Age(years) |                 |     |
| <62       | 20             | 0.3026 |
| ≥62       | 16             |     |
| Unknown   | 5              |     |
| Gender    |                 |     |
| Male      | 24             | 0.5215 |
| Female    | 16             |     |
| Unknown   | 1              |     |
| Tumor size(cm) |         |     |
| >5        | 18             | 0.7139 |
| ≤5        | 23             |     |
| Tumor location |          |     |
| Colon     | 24             | 0.2769 |
| Rectum    | 17             |     |
| Tumor grade |              |     |
| I–II      | 33             | 0.2089 |
| III–IV    | 8              |     |
| Tumor stage |            |     |
| T1–T2     | 10             | 0.6968 |
| T3–T4     | 31             |     |
| Lymph invasion |        |     |
| Yes       | 23             | 0.6311 |
| No        | 17             |     |
| Unknown   | 1              |     |
| Weight loss |             |     |
| Yes       | 20             | 0.2462 |
| No        | 5              |     |
| Unknown   | 16             |     |

*p < 0.05 statistically significant

---

Fig. 3. TPX2 is overexpressed in CRC tissues. Real-time PCR was performed in 41 pairs of CRC tissues and adjacent normal ones (p < 0.0001).
**TPX2 is upregulated in CRC tissues and negatively correlated with miR-485-3p expression**

Recent researches manifested that TPX2 was upregulated in colorectal cancer and involved in CRC progression (42, 43). Our results from Real-time PCR showed that TPX2 mRNA expression level was increased in CRC tissues (Fig. 3). A negative correlation was found between miR-485-3p and TPX2 mRNA expression level by Spearman correlation test ($r = -0.2620$, *P-value: 0.0490)

**miR-485-3p potentially represses AKT pathway via targeting TPX2**

TPX2 plays a vital role in AKT pathway by regulating P53 and P21 mRNA expression level (39). To elucidate whether miR-485-3p can suppress AKT pathway by targeting TPX2, we measured P53 and P21 mRNA expression level and analyzed their correlation with miR-485-3p mRNA expression. We found that downregulation of miR-485-3p can suppress AKT pathway via upregulating TPX2 which leads to downregulation of P21 (Figs 5a and 5b). Although P53 was down regulated in CRC tissues, no association was observed between miR-485-3p and P53 mRNA expression level (Fig. 6).

**miR-485-3p potential role in discriminating between colorectal cancer tissues and their adjacent normal ones**

We used the ROC curve analysis to survey miR-485-3p mRNA expression level effect on discriminating between CRC tissues and their adjacent non-cancerous ones. ROC curve analysis yielded an AUC (the areas under the curve) of 0.9512 (95% CI: 0.8853 to 1.000) to discriminate CRC tissues and adjacent non-cancerous tissues (Fig. 7). An AUC > 0.9 indicates excellent ability of a marker to discriminate two groups of samples.
Fig. 8. Schematic plot of miR-485-3p signaling pathway and its underlying molecules.

Discussion

Colorectal cancer is one of the main causes of cancer-related deaths globally. It is a heterogeneous disease which arises from a constant multistep process that takes at least 10 years and can be detectable at an early stage (1–6). CRC is a suitable option for screening because of its poor prognosis, low survival rate, and high incidence rate (1). In disease screening, biomarkers such as DNA, RNA, microRNA, protein, and antibody are used to assess the disease progression (44). Recent studies illustrated that miRNAs can be recognized as putative biomarkers for their involvement in cancer progression via alteration in their expression profiles (11–14). Currently, emerging evidences proved that dysregulation of miR-485-3p is significantly related to cancer progression and invasion and can be a potential target in cancer therapy. miR-485-3p functions as a tumor-suppressor in different types of cancers and signaling pathways. For example, low expression of miR-485-3p was observed in osteosarcoma that negatively regulate CtBP1, a transcription co-repressor, which can associate with epigenetic enzymes for regulating downstream genes like Bax, Bim, E-cadherin, PUMA, p16, p21, and PTEN (22). Decreased expression of miR-485-3p was clarified to be age-dependent in lung adenocarcinoma (24). In breast cancer, downregulation of miR-485-3p could directly upregulate PGC-1α, transcription co-activator, which contributes to the enhancement of oxidative phosphorylation, mitochondrial biogenesis, and oxygen consumption and as a result provides enough energy for migration and invasion of cancer cells (26). In contrast, miR-485-3p could also exert an effect as oncogene in various cancers. For instance, overexpression of miR-485-3p is determined as a non-invasive biomarker in the peripheral serum of gastric cardia adenocarcinoma patients (16). High expression of miR-485-3p in hepatocellular carcinoma can directly target MAT1A, differentiation marker in liver, and reduce its expression resulting in tumor growth (18). In another study focusing on HCC, miR-485-3p was shown to be upregulated and directly bind to 3'-UTR of NTRK3 which contributed to enhance cell proliferation and invasion (17). Moreover, miR-485-3p could induce EMT via targeting NF-YB at mRNA and protein level in prostate cancer (19, 20).

In the present study, we showed that the expression level of miR-485-3p is significantly downregulated in colorectal cancer tissues. We chose TPX2 as an indirect target of miR-485-3p in CRC. TPX2 is a 100-kDa microtubule-associated protein and plays an important role in mitotic spindle assembly. TPX2 expression is highly controlled during cell-cycle progression and appears in G1-S transition and disappears after cytokinesis completion (29, 32). Previously, upregulation of TPX2 was illustrated in various cancers such as bladder cancer (30), cervical cancer (32) and hepatocellular carcinoma (33). TPX2 is also overexpressed in CRC (44) which is confirmed in our study. Moreover, we surveyed P53 and P21 expression as TPX2 downstream genes and observed a positive correlation between miR-485-3p and P21 expression level. No association between P53 and miR-485-3p expression was found. This can be due to P53 mutation at the late stage of CRC multistep process. P21 correlation with miR-485-3p expression showed a potential effect of miR-485-3p on AKT signaling pathway.

In conclusion, we demonstrated that low expression of miR-485-3p could contribute to TPX2 overexpression and suppress AKT signaling pathway via downregulating P21 in colorectal cancer tissues (Fig. 8). Our study provides novel evidence about miR-485-3p expression and its correlation with TPX2 and P21 expression and recommends miR-485-3p/TPX2/ P21 signaling pathway in CRC for further studies and therapy target for CRC treatment.

References

1. Granados-Romero JJ, Valderrama-Treviño A, Herrera M. Colorectal cancer: a review Clinical review, colorectal cancer View project. Int J Res Med Sci 2017; 5 (11): 4667–4676.
2. Rafieiankach et al. Colorectal cancer in Iran: Epidemiology and morphology trends. EXCLI J 2016; 15: 738–744.
3. Mustafa M, Menon J, Shah MJ, Sharifa AM. Colorectal Cancer: Pathogenesis, Management and Prevention. IOSR J Dent Med Sci e-ISSN 2016; 15 (5): 94–100.
4. Murphy G, Devesa SS, Cross AJ, Inskip PD, McGlynn KA, Cook MB. Sex disparities in colorectal cancer incidence by anatomic subsite, race and age. Int J Cancer 2011; 128 (7): 1668–1675.
5. Boyle P, Leon ME. Epidemiology of colorectal cancer. Br Med Bull 2002; 64 (1): 1–25.
6. Wu WKK, Law PTY, Lee CW et al. MicroRNA in colorectal cancer: from benchtop to bedside. Carcinogenesis 2011; 32 (3): 247–253.
7. Brenner H, Chen C. The colorectal cancer epidemic: challenges and opportunities for primary, secondary and tertiary prevention. Br J Cancer 2018; 119 (7): 785–792.
8. Osada H, Takahashi T. MicroRNAs in biological processes and carcinogenesis. Carcinogenesis 2007; 28 (1): 2–12.
9. Iorio M V, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med 2012; 4 (3): 143–159.
10. Xiong GB, Zhang GN, Xiao Y, Niu BZ, Qiu HZ, Wu B et al. MicroRNA-219-5p functions as a tumor suppressor partially by targeting platelet-derived growth factor receptor alpha in colorectal cancer. Neoplasma. Cancer Research Institute Slovak Acad. of Sciences 2015; 62: 855–63.
11. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6 (11): 857–866.
27. Bellutti S, Basile V, Benatti P, Ferrari E, Marverty G, Imbrioni C. Concurrent inhibition of enzymatic activity and NF-Y-mediated transcription of Topoisomerase-IIa by bis-DemethoxyCurcumin in cancer cells. Cell Death Dis 2013; 4 (8): e756.

28. Brizova H, Kalinova M, Krskova L, Mrhalova M, Kodet R. A novel quantitative PCR of proliferation markers (Ki-67, topoisomerase I, and TPX2): an immunohistochemical correlation, testing, and optimizing for mantle cell lymphoma. Virchows Arch 2010; 456 (6): 671–679.

29. Warner SL, Stephens BJ, Nwokenkwo S et al. Validation of TPX2 as a potential therapeutic target in pancreatic cancer cells. Clin Cancer Res 2009; 15 (21): 6519–6528.

30. Yan L, Li S, Xu C et al. Target protein for Xklp2 (TPX2), a microtubule-related protein, contributes to malignant phenotype in bladder carcinoma. Tumor Biol 2013; 34 (6): 4089–4100.

31. Hsu CW, Chen YC, Su HH et al. Targeting TPX2 Suppresses the Tumorigenesis of Hepatocellular Carcinoma Cells Resulting in Arrested Mitotic Phase Progression and Increased Genomic Instability. J Cancer 2017; 8 (8): 1378–1394.

32. Cheng J, Wang J, Tian Y, Xu J, Gou X, Cheng J. The TPX2 gene is a promising diagnostic and therapeutic target for cervical cancer. Oncol Rep 2012; 27 (5): 1353–1359.

33. Huang Y, Guo W, Kan H. TPX2 is a prognostic marker and contributes to growth and metastasis of human hepatocellular carcinoma. Int J Mol Sci 2014; 15 (10): 18148–18161.

34. Yang X, Liu G, Xiao H et al. TPX2 Overexpression in Medullary Thyroid Carcinoma Mediates TT Cell Proliferation. Pathol Oncol Res 2014; 20 (3): 641–648.

35. Shao C, Duan C, Wang J et al. Expression of microtubule-associated protein TPX2 in human gastric carcinoma and its prognostic significance. Cancer Cell Int 2013; 16 (1): 79.

36. Chen CF, He X, Arslan AD et al. Novel Regulation of Nuclear Factor-YB by miR-485-3p Affects the Expression of DNA Topoisomerase IIA and Drug Responsiveness. Mol Pharmacol 2011; 79 (4): 735–741.

37. Mizuno K, Mataki H, Arai T et al. MiR-154, miR-298, miR-376a, miR-376c, miR-377, miR-381, miR-487b, miR-485-3p, miR-495 and miR-654-3p, mapped to the 14q32.31 locus, regulate proliferation, apoptosis, migration and invasion in metastatic prostate cancer cells. Oncogene 2014; 33 (44): 5173–5182.

38. Luco S, Rainaldi G, Evangelista M, Rizzo M. Fludarabine treatment favors the retention of miR-485-3p by prostate cancer cells: implications for survival. Mol Cancer 2013; 12 (1): 52.

39. Formosa A, Markert EK, Lena AM et al. MicroRNAs, miR-154, miR-299-5p, miR-376a, miR-376c, miR-377, miR-381, miR-487b, miR-485-3p, miR-495 and miR-654-3p, mapped to the 14q32.31 locus, regulate proliferation, apoptosis, migration and invasion in metastatic prostate cancer cells. Oncogene 2014; 33 (44): 5173–5182.

40. Lucotti S, Rainaldi G, Evangelista M, Rizzo M. Fludarabine treatment favors the retention of miR-485-3p by prostate cancer cells: implications for survival. Mol Cancer 2013; 12 (1): 52.

41. Cheng J, Wang J, Tian Y, Xu J, Gou X, Cheng J. The TPX2 gene is a promising diagnostic and therapeutic target for cervical cancer. Oncol Rep 2012; 27 (5): 1353–1359.

42. Chen M, Zhang H, Zhang G et al. Targeting TPX2 suppresses proliferation and promotes apoptosis via repression of the PI3K/AKT/P21 signaling pathway and activation of p53 pathway in breast cancer. Biochem Biophys Res Commun 2018; 507 (1–2): 349–356.

43. Li J, Tian F, Li D et al. MiR-605 represses PSMD10/Gankyrin and inhibits intrahepatic cholangiocarcinoma cell progression. FEBS Lett 2014; 588 (18): 3491–3500.

44. Roy S, Kaur M, Agarwal C, Tecklenburg M, Selafani RA, Agarwal R. p21 and p27 induction by silibin is essential for its cell cycle arrest effect in prostate carcinoma cells. Mol Cancer Ther 2007; 6 (10): 2696–2707.

45. Wei P, Zhang N, Xu Y et al. TPX2 is a novel prognostic marker for the growth and metastasis of colon cancer. J Transl Med 2013; 11: 313.

46. Langan RC, Mullinax JE, Raiji MT et al. Colorectal cancer biomarkers and the potential role of cancer stem cells. J Cancer 2013; 4 (3): 241–250.