A Review of Ribonucleotide Reductase and Cancer Therapies

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Abstract

Mammalian Ribonucleotide reductase (RNR) provides the precursors needed for both DNA synthesis and repair process. Recent study shows RNR including RRM2 have been associated with various types of cancer and many studies imply that it plays biological roles in promoting cancer development. The important role plays by RNR, and its subunits RRM1, RRM2, and p53R2 in DNA synthesis and repair had found to be an attractive target for anticancer therapies. Usually targeting RNR in cancer treatment using known inhibitors, or new inhibitors that discovered using as molecular docking programs. This comprehensive strategy can be used to find new indications of clinically used drugs that failed during its development. Thus, this strategy has the advantages of cost reduction and bypasses the safety concerns by in silico evaluation of the inhibitor biological activity on the molecular target.

This review compiles studies on the structure, function, and regulation activity of RNR in cancer; the role of RNR in cancer development and the strategy of RNR inhibition for future RNR inhibitor discovery in cancer treatment.

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Introduction

RNR activity is highly associated with cellular proliferation. An increased expression and activity of RNR in mammalian cell are associated with malignant transformation and cancer development.[1] RRM2 overexpression is associated with the development of cancer cells. This review paper discusses mainly on RNR expression and cancer, as well as its role in cancer therapies.

RNR expression and regulation activity

The preservation of a balanced dNTP pool is a prerequisite for high constancy DNA replication and restore following the DNA damage.[2] Therefore, all classes of RNRs are subjected to multiple modes of regulation as shown in Table 1.

However, due to the conserved mechanisms in mammalian cell, RNR are regulated through allosteric and oligomeric regulations, as well as changes of the level and localization of RNR subunits.[3] RNR accomplish allosteric modulation through allosteric mechanism which requires the coordination of two allosteric effector binding sites and the catalytic site.[4] RNR expression is inhibited if there is a high concentration of dATP.[5] Thus, the relative concentrations of ATP and dATP are important in regulating RNR activity.

No	Modes of regulation	References
1.	Allosteric regulation	2,6
2.	Subunit oligomerization	7,8
3.	Transcriptional regulation of RNR genes	9
4.	Binding of small protein inhibitors	10
5.	Subunit compartmentalization	11

Table 1. Multiple modes of regulation in RNR.

Ribonucleotide Reductase in Cancer

A complex series of cellular and molecular changes that occur during cancer

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development can be facilitated by diverse endogenous stimuli.[12] It can be detected by various biological fluids including saliva.[13] During cancer development, uncoordinated cell proliferation can leads to a distorted level and unbalance of dNTPs, which will then cause replication stress and further promote genomic instability.[4] Uncontrolled proliferation of cancer cells must be supported by sufficient amount of dNTP supply. Hence, cancer cells require increased RNR activity to support the demand for dNTPs that is needed for cancer cells rapid proliferation.[4]

RNR has been associated with various types of cancer in many studies.[14,15] The accumulating evidences which imply that RNR subunits play biological roles in promoting cancer development, it also acts as dNTPs synthesizer.[1] Opposing regulation of RRM2 and p53R2 could have the potential in determining the invasion and metastatic phenotype in cancer cells [16]. Studies have shown that alteration of RRM1 and RRM2 expression balance can modulate transformation, tumourigenicity,[17] and metastatic potential.[18] This is one of the factors that may lead to mutagenesis, carcinogenesis and even cell death.

Several studies suggested that RRM1 helps in the repair mechanism of altered dNTP pool which protects cancer cells against lethal stresses caused by genotoxic chemotherapies treatment.[14,19] This could suggest the complex and stage-specific roles of RRM1 in tumourigenesis.[1] There are several studies which proposed RRM1 as a tumour initiation suppressor. RRM1 overexpression in cultured cells leads to reduced transformation and suppression of lung tumourigenicity and metastasis.[20]

Nevertheless, the role of RRM1 in cancer still remains controversial. This is because, in early stage of non-small-cell lung cancer (NSCLC), a high level of RRM1 expression along with ERCC1 or PTEN up-regulation is a determinant for optimal survival rate.[21–23] RRM1 overexpression results in a poor outcome as it is proposed to have relation in resistance to gemcitabine in NSCLC and pancreatic cancer.[14,19] These studies indicate that even in the same cancer type at different stages or under different therapies, RRM1 overexpression may results in contradictory different outcome.

Different from the tumour suppressing role of RRM1, RRM2 has been proposed to be associated with tumour inducing activity.[17] With the use of ONCOMINE database, RNR gene expression in human cancers was surveyed by study done by Aye et al. (2015). It was observed notably that among the RNR genes, RRM2 gene was the most overexpressed genes in most of cancer analyses, which includes sarcoma and cancers of the bladder, brain and central nervous system, colorectal, breast, lung and liver.[4] In contrast, RRM1 and p53R2 gene were overexpressed in several type of cancer analysis.[4]

RRM2 was investigated to be overexpressed in gastric, ovarian, bladder and colorectal cancers.[15,24–26] In both breast and epithelial ovarian cancers, RRM2 expression is seen to be correlated with tumour grade.[24,27] This may suggest a role of RNR in assisting rapid cell division of high-grade tumours. Besides that, it was also proposed that RRM2 assist in oncogenic activity of cancer cells. In a study done by Fan et al. (1998), it was observed that RRM2 and oncoproteins cooperate with each other to increase formation and anchorage independent growth in mouse cells.[28] Similarly, RRM2 overexpression also increases cellular invasiveness and expression of the metalloprotease MMP-9,[29] which associates with solid tumour malignancies in prostate cancer.[30]

Besides that, overexpression of RRM2 increases pancreatic adenocarcinoma cellular aggressiveness and MMP-9 expression in a NF-κB-dependent manner.[29] Overexpression of RRM2 could act as an activator of the Ras/Raf pathway in promoting cell proliferation and invasiveness, which results in recurrence of prostate cancer.[29,31] In studies done by Huang *et al.* (2014), found that RRM2 knockdown in PC-3 and LNCap cells reduces MMP-9 and Ras activity. [30] These findings suggest that RRM2 protein also can cooperate with several oncogenes, including v-fms,v-src,A-raf,v-fes,c-myc, and ornithine decarboxylase to promote tumourigenesis and transformation.[32]

Similar to the contradictory results of RRM1 in the development of cancer cell, the role of p53R2 in cancer cell development is also controversial. p53R2 and RRM1 has similar biological features and possess functions in metastasis-suppression in colorectal cancer.[33] Tumour suppressor gene p53 target directly to p53R2 during DNA repair mechanism, for

assisting in DNA damage repair during G2 arrest and in addition to provides DNA precursors.[34,35] Hence, malfunctioning of p53R2 could result in catastrophe DNA damage repair mechanism and thus leads to mutation of the gene or even activation of cellular apoptosis.[36] The p53R2 is proposed to inhibit malignancy by protecting cells from oxidative damage by eliminating free radicals.[1] Besides that, high level of p53R2 expression also suppresses cancer cell proliferation, independent of p53 by upregulating p21 and downregulating cyclin D1.[37]

However, an overexpression of p53R2 has been reported in melanoma, oral carcinoma, esophageal squamous cell carcinoma and NSCLC.[38,39] High level of p53R2 expression has been significantly associated with stage, invasiveness, metastasis and poor prognosis for patients who had esophageal squamous cell carcinoma and oral cancer.[40] The p53R2 cooperates with MEK2 and promotes cancer cell invasion and metastasis by negatively regulates the Ras-MEK-ERK pathway.[41] Overexpression of p53R2 has been linked with the longer survival rate of colorectal cancer patients, who later had tumour size, lymph nodes and metastasis (TNM) stage cancers.[33,41]

Ribonucleotide Reductase and Cancer Therapies

Statistically, cancer cases such as colocteral cancer, pancreatic cancer, breast cancer, leukemia and, oral cancer were estimate increasing each year worldwide.[42] Thus, approaches of new surgical techniques, adjuvant therapy and molecular targeted therapy are urgently needed as to increase the number of cancer patient survivals. RNR has an essential role in converting ribonucleoside diphosphate to 2-deoxyribonucleoside diphosphate to maintain homeostasis of nucleotide pools. The important role played by RNR in DNA synthesis and repair had found to be an attractive target for anticancer agents. Increased of RNR activity is associated with malignant transformation and tumour cell growth which suggest that the inhibition of RNR might have a potential to treat cancers. Therefore, the potential role of RNR in cancer treatment RNR and its subunits RRM1, RRM2, and p53R2 are key factors in supplying dNTP and hence, are considered as major targets in cancer therapies.[43–45]

Inhibitors of Ribonucleotide Reductase

RNR represents an important target for cancer therapy. There are only three RNR inhibitors in clinical use: hydroxyurea (HU), 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine), and GT12040. HU acts to block DNA synthesis by reducing the tyrosyl free radical. It has also been marketed as cancer therapeutics for many years and the only RNR inhibitor that is commercially available.[46] Nevertheless, resistance to HU treatment is a common problem, 3-AP, a small molecule iron chelator inactivates RNR, has been found to cause hypoxia, respiratory distress and mathemoglobulin of red blood cells.[47] GTI2040 is an antisense molecule which was tested to be ineffective in human trials.[48] Furthermore, other issues relating to these three inhibitors are incomplete RNR blocking, short half-life, and regeneration of RNR.[49] Therefore, an ideal RNR inhibitor for use in cancer therapy would have greater potency than HU, less iron chelating ability 3-AP and has specific target.

In addition, other inhibitors of RNR enzyme also have been reported in molecular docking studies such as Flavin (Fla) and Phenosafranine (Phe). The docked inhibitors, Flavin and Phenosafranine binds at the active cite TYR176, which are essential for free radical formation [50]. This mechanism helps to understand the functional aspects of R2. An increased level of RNR produces more DNA, and thus drives the cells through DNA synthesis. In a hypoxic environment where the activity of RNR is stress out by lack of oxygen, the level of RNR regulates the production of DNA and drives the cells even faster through DNA synthesis.[50] This implies that the replication machinery in the cells is able to detect the level of DNA, when exogenous DNA is supplied.

An inhibitor, CHO29 also has been tested in vivo where it showed potential characteristics for inhibits cancer cell growth as well as overcome drug resistance. COH29 is a small-molecule which acts as interference with RNR subunits and radical transfer, disturbance of the iron centre, and blocking the oxygen passage channel. Therefore, it disrupts holoenzyme of complex formation and inhibits the cancer cell progression.[49]

On the other hand, in vitro studies also have been carried out on RNR inhibitors such as Gemcitabine (GEM), Cisplatin (CDDP) and 5-Fluoroucil. CDDP-based chemotherapy is generally the first-line treatment for inoperable

recurrent or metastatic in oral cancer. In the study done by Iwamoto *et al.* (2015), they indicated that GEM had more potent antitumour activity against oral cancer cell than CDDP and 5-FU.[44] However, the mixed of two drugs, CDDP and 5-FU (PF) in chemotherapy is the most widely used therapy in the treatment of patients with oral cancer.

GEM, triapine, and GTI-2040 are also known as RRM2 inhibitors.[51] Triapine and GTI-2040 have been evaluated in phase I clinical trials.[52,53]

The relationship between RRM2 mRNA expression levels and the response to GEM in the clinical setting has been investigated in various cancers. In pancreatic cancer, the response rate to GEM is significantly higher in patients with low RRM2 mRNA expression.[54] Furthermore, patients with lung adenocarcinoma with low levels of RRM2 mRNA are responded to GEM treatment.[55] Those facts indicated the possibility of RRM2 mRNA expression levels could predict chemosensitivity to GEM.

In addition, they also found RRM2 plays a critical role in supporting the growth of human oral cancer cells, and agents targeting RRM2, such as GEM, appear to be a potentially useful therapeutic approach for oral cancer. However, these inhibitors for RNR enzyme still not tested on clinical trial. Further steps are still needed to retrieve inhibitor activity for achieving immediate and good therapy for cancer treatment. **Table 2** shows list of current inhibitors for RNR enzyme.

No.	Inhibitors	Methods	References
1.	Triapine or thiosemicarbazone (TSC)	Phase 1 clinical trial	52
2.	GTI-2040	Phase 1/2 clinical trial	53
3.	Hydroxyurea (HU)	Clinical trial	47
5.	Flavin	In silico	50
6.	Phenosafranine	In silico	50
7.	COH29	In Vivo	49
8.	Gemcitabine (GEM)	In Vitro	54
9.	Cisplatin (CDDP)	In Vitro	54
10.	5-fluorouracil	In Vitro	54

Table 2. Current drugs /inhibitors available for RNR.

Conclusions

RNR is one of the most highly conserved and regulated enzyme which has a fundamental and essential function for DNA biosynthesis. Recent advancement in RNR holoenzyme structure modelling and regulation mechanisms have significantly excavated our understanding and knowledge about this enzyme in various cancer development. In addition to RNR fundamental function to supply dNTPs, each of the individual three subunit proteins (RRM1, RRM2 and p53R2) plays other enzyme independent roles in cancer development. RNR plays an important role for cancer therapy.

Therefore, a comprehensive molecular docking program has advantages in the reduction of costs and the bypass of safety concerns by in silico evaluation of the inhibitor biological activity on the molecular target should be considered to find new indications of clinically used drugs or compounds that failed during its development. Thus, it is not only required a public educational awareness,[56] but also a very comprehensive drug screening program to fighting against cancer.

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Declaration of Interest

All authors declare no competing interest.

References

1. Shao J, Liu X, Zhu L, Yen Y. Targeting ribonucleotide reductase for cancer therapy. Expert opinion on therapeutic targets. 2013;17(12):1423-37.
2. Jordan A, Reichard P. Ribonucleotide reductases. Annual review of biochemistry. 1998;67(1):71-98.
3. Wijerathna SR, Ahmad MF, Xu H, et al. Targeting the Large Subunit of Human Ribonucleotide Reductase for Cancer Chemotherapy. Pharmaceuticals. 2011;4(10):1328-54.
4. Aye Y, Li M, Long MJC, Weiss RS. Ribonucleotide reductase and cancer: biological mechanisms and targeted therapies. Oncogene. 2015;(April 2014):2011-21.
5. Fairman JW, Wijerathna SR, Ahmad MF, et al. Structural basis for allosteric regulation of human ribonucleotide reductase by nucleotide-induced oligomerization. Nature structural & molecular biology. 2011;18(3):316-22.
6. Kashlan OB, Scott CP, Lear JD, Cooperman BS. A comprehensive model for the allosteric regulation of mammalian ribonucleotide reductase. Functional consequences of ATP-and dATP-induced oligomerization of the large subunit. Biochemistry. 2002;41(2):462-74.
7. Mulder KW, Winkler GS, Timmers HTM. DNA damage and replication stress induced transcription of RNR genes is dependent on the Ccr4–Not complex. Nucleic Acids Res. 2005;33(19):6384-92.
8. Rofougaran R, Crona M, Vodnala M, Sjöberg B-M, Hofer A. Oligomerization status directs overall activity regulation of the Escherichia coli class Ia ribonucleotide reductase. Journal of Biological Chemistry. 2008;283(51):35310-18.

9. Chabes A, Georgieva B, Domkin V, Zhao X, Rothstein R, Thelander L. Survival of DNA damage in yeast directly depends on increased dNTP levels allowed by relaxed feedback inhibition of ribonucleotide reductase. Cell. 2003;112(3):391-401.
10. Ainsworth WB, Hughes BT, Au WC, et al. Cytoplasmic localization of Hug1p, a negative regulator of the MEC1 pathway, coincides with the compartmentalization of Rnr2p–Rnr4p. Biochem Biophys Res Commun. 2013;439(4):443-8.
11. Stubbe J. Di-iron-tyrosyl radical ribonucleotide reductases. Current opinion in chemical biology. 2003;7(2):183-8.
12. Fan H, Villegas C, Wright JA. Ribonucleotide reductase R2 component is a novel malignancy determinant that cooperates with activated oncogenes to determine transformation and malignant potential. Proceedings of the National Academy of Sciences. 1996;93(24):14036-40.
13. Laidi F, Zaoui F. Saliva diagnostic and cancer monitoring : Overview. Journal of International Dental and Medical research. 2015;8(2):94-97.
14. Cao MY, Lee Y, Feng NP, et al. Adenovirus-mediated ribonucleotide reductase R1 gene therapy of human colon adenocarcinoma. Clin Cancer Res. 2003;9(12):4553-61.
15. Morikawa T, Maeda D, Kume H, Homma Y, Fukayama M. Ribonucleotide reductase M2 subunit is a novel diagnostic marker and a potential therapeutic target in bladder cancer. Histopathology. 2010;57(6):885-92.
16. Furuta E, Okuda H, Kobayashi A, Watabe K. Metabolic genes in cancer: Their roles in tumor progression and clinical implications. Biochim Biophys Acta - Rev Cancer. 2010;1805(2):141-152.
17. Khor GH, Ruth G, Froemming A, Zain RB, Lin TK. Aberrant Methylation of Ribonucleotide Reductase Subunit M2 Is Closely Associated with Oral Cancer. Academic Journal of Cancer Research. 2016;9(4):63-69.
18. Elford L, Freese M, Passamani E, Morris HP. Ribonucleotide Reductase and Cell Proliferation. The Journal of Biological Chemistry. 1970;245(20):5238-43.
19. Lee JJ, Maeng CH, Baek SK, et al. The immunohistochemical overexpression of ribonucleotide reductase regulatory subunit M1 (RRM1) protein is a predictor of shorter survival to gemcitabine-based chemotherapy in advanced non-small cell lung cancer (NSCLC). Lung Cancer. 2010;70(2):205-10.
20. Gautam A, Li Z-R, Bepler G. RRM1-induced metastasis suppression through PTEN-regulated pathways. Oncogene. 2003;22(14):2135-42.
21. Davidson JD, Ma L, Flagella M, Geeganage S, Gelbert LM, Slapak CA. An increase in the expression of ribonucleotide reductase large subunit 1 is associated with gemcitabine resistance in non-small cell lung cancer cell lines. Cancer Res. 2004;64(11):3761-66.
22. Zhang K, Wu J, Wu X, et al. p53R2 inhibits the proliferation of human cancer cells in association with cell-cycle arrest. Molecular cancer therapeutics. 2011;10(2):269-78.
23. Souglakos J, Boukovinas I, Taron M, et al. Ribonucleotide reductase subunits M1 and M2 mRNA expression levels and clinical outcome of lung adenocarcinoma patients treated with docetaxel/gemcitabine. British journal of cancer. 2008;98(10):1710-15.
24. Liu X, Zhang H, Lai L, et al. Ribonucleotide reductase small subunit M2 serves as a prognostic biomarker and predicts poor survival of colorectal cancers. Clinical science. 2013;124(9):567-78.
25. Lu AG, Feng H, Wang PXZ, Han DP, Chen XH, Zheng MH. Emerging roles of the ribonucleotide reductase M2 in colorectal cancer and ultraviolet-induced DNA damage repair. World Journal of Gastroenterology. 2012;18(34):4704-13.
26. Khor GH, Ruth G, Froemming A, Zain RB, Abraham MT, Thong KL. Screening of Differential Promoter Hypermethylated Genes in Primary Oral Squamous Cell Carcinoma. Asian Pacific Journal of Cancer Prevention. 2014;15:8957-61.
27. Kunz BA, Kohalmi SE. Modulation of mutagenesis by deoxyribonucleotide levels. Annual review of genetics. 1991;25(1):339-59.
28. Fan H, Villegas C, Huang A, Wright J a. The Mammalian Ribonucleotide Reductase R2 Component Cooperates with a Variety of Oncogenes in Mechanisms of Cellular Transformation The Mammalian Ribonucleotide Reductase R2 Component Cooperates with a Variety of Oncogenes in Mechanisms of Cellular Transf. Cancer Res. 1998;58(204):1650-53.
29. Illemann M, Bird N, Majeed A, et al. MMP-9 Is Differentially Expressed in Primary Human Colorectal Adenocarcinomas and Their Metastases. Molecular Cancer Research. 2006;4(May):293-302.
30. Huang Y, Liu X, Wang YH, et al. The prognostic value of ribonucleotide reductase small subunit M2 in predicting recurrence for prostate cancers. Urologic Oncology: Seminars and Original Investigations. 2014;32(1):51.e9-51.e19.
31. Khor GH, Froemming GA, Tan AC. et al. Pathways Deregulation in Oral Squamous Cell Carcinoma using Methylation Profiling. Journal Dental Research. 2011;91(Special issue C): abs47.
32. Liu X, Zhou B, Xue L, et al. Metastasis-suppressing potential of ribonucleotide reductase small subunit p53R2 in human cancer cells. Clin Cancer Res. 2006;12(21):6337-44.
33. Nakano K, Bálint E, Ashcroft M, Vousden KH. A ribonucleotide reductase gene is a transcriptional target of p53 and p73. Oncogene. 2000;19:4283-89.
34. Yamaguchi T, Matsuda K, Sagiya Y, et al. p53R2-dependent Pathway for DNA Synthesis in a p53-regulated Cell Cycle Checkpoint. Cancer Res. 2001;61:8256-62.
35. Devlin H-L, Mack PC, Burich R a, et al. Impairment of the DNA repair and growth arrest pathways by p53R2 silencing enhances DNA damage-induced apoptosis in a p53-dependent manner in prostate cancer cells. Molecular cancer research : MCR. 2008;6(5):808-18.
36. Xue L, Zhou B, Liu X, et al. Ribonucleotide reductase small subunit p53R2 facilitates p21 induction of G1 arrest under UV irradiation. Cancer Res. 2007;67(1):16-21.
37. Matsushita S, Ikeda R, Fukushige T, et al. P53R2 is a prognostic factor of melanoma and regulates proliferation and chemosensitivity of melanoma cells. Journal of Dermatological Science. 2012;68(1):19-24.
38. Hsu N, Wu J, Liu X, et al. Expression Status of Ribonucleotide Reductase Small Subunits hRRM2 / p53R2 as Prognostic Biomarkers in Stage I and II Non-small Cell Lung Cancer. Anticancer Res. 2011;3482(110):3475-81.
39. Okumura H, Natsugoe S, Yokomakura N, et al. Expression of p53R2 Is Related to Prognosis in Patients with Esophageal Squamous Cell Carcinoma. Clin Cancer Res. 2006;12(12):3740-45.
40. Liu X, Lai L, Wang X, et al. Ribonucleotide reductase small subunit M2B prognoses better survival in colorectal cancer. Cancer Res. 2011;71(9):3202-13.
41. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1. 0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11. 2013. International Agency for Research on Cancer. 2014.
42. Priya PL, Shanmughavel P. A docking model of human ribonucleotide reductase with flavin and phenosafranine. Bioinformation. 2009;4(3):123-26.
43. Iwamoto K, Nakashiro K-I, Tanaka H, Tokuzen N, Hamakawa H. Ribonucleotide reductase M2 is a promising molecular target for the treatment of oral squamous cell carcinoma. International journal of oncology. 2015;46(5):1971-1977.
44. Cho E-C, Yen Y. Novel regulators and molecular mechanisms of p53R2 and its disease relevance. Biochimie. 2016;123:81-84.
45. Nyholm S, Thelander L, Gräslund A. Reduction and loss of the iron center in the reaction of the small subunit of mouse ribonucleotide reductase with hydroxyurea. Biochemistry. 1993;32(43):11569-74.
46. Yen Y, Margolin K, Doroshow J, et al. A phase I trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone in combination with gemcitabine for patients with advanced cancer. Cancer chemotherapy and pharmacology. 2004;54(4):331-42.

47. Leighl NB, Laurie SA, Chen XE, et al. A phase I/II study of GTI-2040 plus docetaxel as second-line treatment in advanced non-small cell lung cancer: a study of the PMH phase II consortium. Journal of Thoracic Oncology. 2009;4(9):1163-69.
48. Zhou B, Su L, Hu S, et al. A small-molecule blocking ribonucleotide reductase holoenzyme formation inhibits cancer cell growth and overcomes drug resistance. Cancer Res. 2013;73(21):6484-6493.
49. Natarajan S, Mathews R. Modeling and proposed mechanism of two radical scavengers through docking to curtail the action of ribonucleotide reductase. Journal of Biophysics and Structural Biology. 2011;3(2):38–48.
50. Shao J, Zhou B, Chu B, Yen Y. Ribonucleotide reductase inhibitors and future drug design. Current cancer drug targets. 2006;6:409-31.
51. Wadler S, Makower D, Clainmont C, Lambert P, Fehn K, Sznol M. Phase I and pharmacokinetic study of the ribonucleotide reductase inhibitor, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone, administered by 96-hour intravenous continuous infusion. Journal of Clinical Oncology. 2004;22(9):1553-63.
52. Desai A, Schilsky R, Young A, et al. A phase I study of antisense oligonucleotide GTI-2040 given by continuous intravenous infusion in patients with advanced solid tumors. Annals of Oncology. 2005;16(6):958-65.
53. Itoi T, Sofuni A, Fukushima N, et al. Ribonucleotide reductase subunit M2 mRNA expression in pretreatment biopsies obtained from unresectable pancreatic carcinomas. Journal of Gastroenterology. 2007;42(5):389-94.
54. Morris GM, Goodsell DS, Huey R, Olson AJ. Distributed automated docking of flexible ligands to proteins: parallel applications of AutoDock 2.4. Journal of computer-aided molecular design. 1996;10(4):293-304.
55. Morikawa T, Hino R, Uozaki H, et al. Expression of ribonucleotide reductase M2 subunit in gastric cancer and effects of RRM2 inhibition in vitro. Human Pathology. 2010;41(12):1742-1748.
56. Natheer H Al-Rawi, Sausan Al-Kawas, Omer I. Public awareness and attitude toward oral cancer screening in United Arab Emirates. Journal of International Dental and Medical Research. 2012; 5 (3): 149-154.