EMODIN DOWNREGULATES CELL PROLIFERATION MARKERS DURING DMBA INDUCED ORAL CARCINOGENESIS IN GOLDEN SYRIAN HAMSTERS

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Abstract

Background: Cell-cycle disruption is the major characteristic features of neoplastic transformation and the status of cell-cycle regulators can thus be utilized to assess the prognostic significance in patients with cancer. The PCNA, cyclin D1, CDK4, CDK6 and survivin expression in the buccal mucosa was utilized to evaluate the Emodin efficacy on abnormal cell proliferation during 7,12-dimethylbenz(a)anthracene (DMBA) induced oral carcinogenesis in golden Syrian hamsters.
Materials and methods: Topical application of DMBA, three times a week for 14 weeks, on the hamsters' buccal pouches developed well differentiated squamous cell carcinoma.
Results: Cyclin D1 and PCNA over-expression and up-regulation of CDK4, CDK6 and survivin were noticed in the buccal mucosa of hamsters treated with DMBA alone. Emodin administration (50mg/kg b.w) orally to hamsters treated with DMBA down-regulated the expression of cell proliferation markers in the buccal mucosa.
Conclusions: The anti-cell proliferative role of Emodin is owing to its modulating efficacy on cell-cycle markers towards the tumor suppression during DMBA induced oral carcinogenesis.

Keywords: Oral carcinogenesis, Emodin, Hamsters, DMBA

Introduction

Cancer, a silent killer of the human life as well as a major threat to the public health, is characterized by rapid and uncontrolled growth, invasion and metastasis (Kwon, 2013).

World Health Organization in its recent report pointed out a fourteen million newly diagnosed cancer cases and 8.2 million oral cancer related deaths by the year 2012 worldwide (Siegel et al., 2016). Oral carcinoma imposes a significant health burden, morbidity and mortality worldwide (Siddiqi et al., 2015).

While various histological types of oral cancers are recognized, squamous cell carcinoma constitutes around 90% of all oral carcinomas (Gondak et al., 2012). The incidence of oral carcinoma is increasing every year in several parts of the world due to widespread habits of tobacco and alcohol abuse (Maaly et al., 2015).

Worldwide, around 300,000 new oral cancer cases and 145,000 deaths due to oral carcinoma were reported in the year 2012 (Torre et al., 2015). India reports the highest incidence of oral cancer every year, where 75,000-80,000 Indians are affected by this cancer every year (Hebbar et al., 2014).

7,12-dimethylbenz(a)anthracene is commonly utilized as a major carcinogen to develop tumors in the golden Syrian hamsters' buccal pouches (Karthikeyan et al., 2013).

Buccal pouches, a pocket like anatomy, exposed to repeated topical applications of DMBA resulted in well developed oral squamous cell carcinoma. DMBA causes neoplasm by inducing severe inflammation and dysplasia in the buccal pouches as well as by causing extensive oxidative damage to DNA (Manoharan et al., 2016).

Accumulated evidences pointed out histological, morphological, biochemical and molecular similarities between DMBA induced oral tumors and human oral tumors (Tanaka and Ishigamori, 2011).

This experimental oral cancer model is therefore utilised as a preferred one to study the tumor preventive potential of natural products and their bioactive constituents.

Cell-cycle disruption is the major characteristic features of neoplastic transformation and the status of cell-cycle regulators can thus be utilized to assess the prognostic significance in patients with cancer. Disturbance in the cell-cycle regulation is the hallmark of carcinogenesis (Velez et al., 2015; Bertoli et al., 2013).

The orderly cell-cycle progression is finely monitored and regulated by cyclins, along with their regulatory enzymes, cyclin dependent kinases. Cyclins and cyclin dependent kinases play an essential role in the cell-cycle regulation (Lim and Kaldis, 2013). Several studies have pointed out cyclin D1 and cyclin dependant kinases as a significant molecular objective for the inhibition of oral carcinogenesis (Manoharan et al., 2015).

PCNA, a 36k Da protein, is located on chromosome 20 and serves as a key role in the late G1→S phase of the cell-cycle. In eukaryotic cells, it proceeds as a cofactor of DNA polymerases (Asghar, et al., 2015).

PCNA is regarded as a cell proliferation marker and has a critical role in DNA repair, DNA replication and chromatin remodelling (Strzalka and Ziemienowicz, 2011; Diamant et al., 2012). Up-regulation of PCNA has been observed in almost all forms of carcinomas (Khalil et al., 2015).

Survivin has multiple biological activities, which includes a regulatory role in mitosis and apoptosis. Survivin performs a key role in the immunity and cell differentiation. Survivin also plays a crucial role in invasion and angiogenesis (Chen et al., 2016; Jaiswal et al., 2015). Survivin has a prognostic significance, as this protein was over-expressed in several tumors, including colon, liver, mammary, bladder and oral carcinoma (Chen et al., 2016).

Emodin is a purgative anthraquinone found in several plants of the genus Rhamnus especially Rhamnus frangula. It is also rich in Rheum emodi, a Himalayan rhubarb, *Rheum palmatum*, *Rheum tanguticum,* and *Ventilago madraspatana*. Emodin is also produced by a lot of fungi, including Aspergillus, Pyrenochaeta, and Pestalotiopsis. Emodin is commonly employed in traditional Chinese medicine for various diseases.

Emodin showed protective effects against insulin resistance, hyperglycemia, obesity and endothelial cell dysfunction. Wang et al., (2011) explored the anticancer potential of Emodin in gallbladder cancer cells. The anti-inflammatory effect of Emodin has been demonstrated in Wistar rats (Chen et al., 2015).

Studies have also been documented the antidiabetic and hepatoprotective potential of Emodin in experimental models (Arvindekar et al., 2015; Bhadauria, 2010).

Xiong et al., (2011) demonstrated the *in vitro* and *in vivo* antiviral effect of Emodin aganist Herpes Simplex Virus. This study utilizes the PCNA, cyclin D1, CDK4, CDK6 and survivin expression to evaluate the effect of Emodin on abnormal cell proliferation occurring during DMBA induced oral carcinoma in the golden Syrian hamsters.

Materials and methods
Animals

For the present study, we purchased forty male golden Syrian hamsters, weighing 80-120g, from National Institute of Nutrition, Hyderabad, India. The experimental hamsters were maintained at the Annamalai University Central Animal House, as per the Institution Ethical Committee principles (Registration Number 160/1999/ CPCSEA). The experimental hamsters were housed in the polypropylene animal cages and the pellet diet and water were provided *ad* libitum.

Tumor induction

Oral tumors were developed in the buccal mucosa of golden Syrian hamsters using topical application of 0.5% 7,12-dimethyl benz(a)anthracene in liquid paraffin (three times a week for 14 weeks).

Experimental design

To assess the anti-cell proliferative efficacy of Emodin, the experimental hamsters were categorized into four groups of ten animals in each as follows:

- Group I: Topical application of liquid paraffin alone (three times a week for 14 weeks)

- Group II   : Topical application of DMBA alone (0.5% in liquid paraffin, three times a week for 14 weeks)

- Group III:Topical application of DMBA (0.5% in liquid paraffin, three times a week for 14 weeks) + Oral administration of Emodin (50mg/kg b.w, three times a week for 14 weeks on alternate days of DMBA application)

- Group IV : Oral administration of Emodin alone (50mg/kg b.w, three times a week for 14 weeks)

Western blotting

After quantification of the protein in the buccal mucosa tissue extract, it was subjected to polyacrylamide gel electrophoresis to separate the various proteins. Then the protein bands were transferred onto PVDF membrane using electroblotting. The blots were then treated with corresponding primary antibodies (CDK4, CDK6 and Survivin, Cell Signaling Technology, Danvers, MA, USA), followed by incubation with secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology, USA). The obtained immune complex was then treated with the enzyme substrate, diaminobenzidine. The protein bands were scanned and analysed densitometrically (Bio-Rad Image Lab™ software version 4.1 software).

Immunohistochemistry

After a routine procedure, the buccal mucosa tissue sections were treated with the corresponding primary antibodies (PCNA and cyclin D1: Dako, Carprinteria, CA, USA), followed by incubation with the horseradish peroxidase labelled secondary antibodies.

The enzyme substrate, diaminobenzidine, was then added and the immune complex formed was viewed under the microscope, when acceptable color intensity was attained.

Statistical analysis

One way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) was done to assess the statistical significance between the two experimental groups. The two groups were considered as statistically significant if the p value was less than 0.05 between them.

**Results**

Tumor incidence

We noticed well-differentiated oral squamous cell carcinoma, confirmed by the oral pathologist, in the buccal mucosa of all the hamsters received topical application of DMBA alone. The tumor incidence was therefore hundred percent. While tumor formation was absent in the hamsters treated with DMBA+Emodin, we noticed precancerous pathological lesions such as hyperplasia, hyperkeratosis and dysplasia (table 1). The present results thus suggest the tumor inhibitory potential of Emodin in DMBA induced oral carcinogenesis.

Cell proliferation markers

The expression pattern of cyclin D1, PCNA (Immunohistochemistry) and CDK4, CDK6 and survivin (Western blotting) in the buccal mucosa of control and experimental hamsters are depicted in the figures 1, 2 and 3 respectively. Over-expression of cyclin D1 and PCNA and up-regulation of CDK4, CDK6 and survivin were observed in the buccal mucosa of hamsters treated with DMBA alone. Emodin administration at a dose of 50mg/kg b.w orally to hamsters treated with DMBA down-regulated the expression of the above mentioned cell proliferation markers in the buccal mucosa.

Table 1: Tumor incidence and histopathological features observed in control and experimental hamsters in each group.

| Parameter	Liquid paraffin alone treated hamsters	DMBA alone treated hamsters	DMBA+ Emodin treated hamsters	Emodin alone treated hamsters
Tumor incidence	0%	100%	0%	0%
Hyperplasia	Not observed	Severe hyperplastic lesions	Mild to moderate hyperplastic lesions	Not observed
Hyperkeratosis	Not observed	Severe hyperkeratotic lesions	Mild to moderate hyperkeratotic lesions	Not observed
Dysplasia	Not observed	Severe dysplastic lesions	Mild to moderate dysplastic lesions	Not observed
Well differentiated squamous cell carcinoma	Not observed	Tumor formation in all the animals	Not observed	Not observed
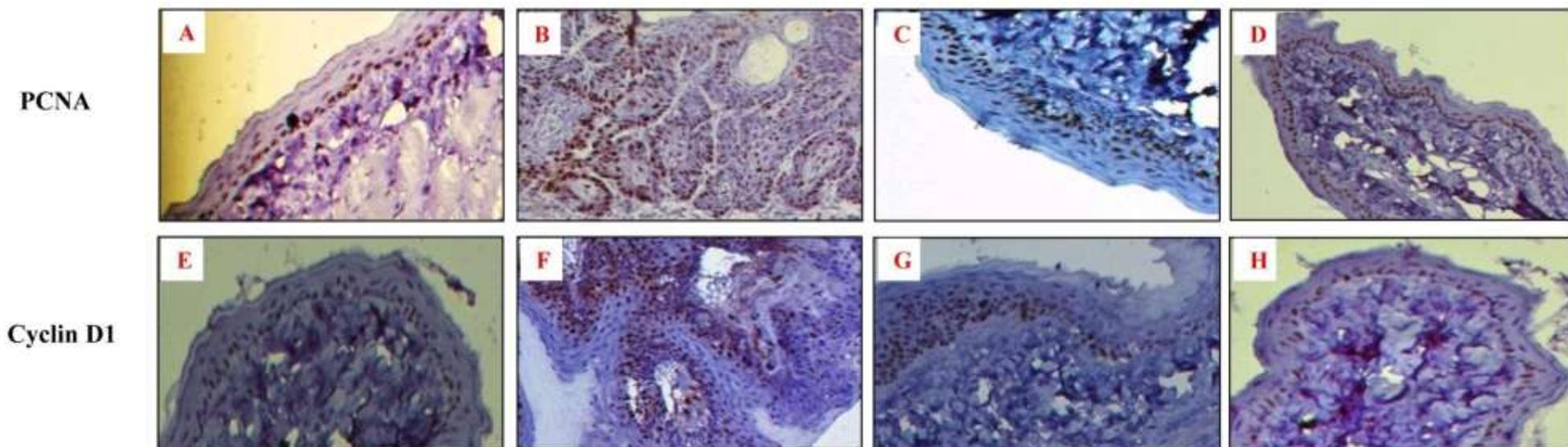
Figure 1: PCNA and Cyclin D1 expression in the buccal mucosa of experimental hamsters
PCNA: A and D - Control and Emodin alone (expression not detectable), B - DMBA alone (over-expressed), C - DMBA + Emodin (down-regulated)
Cyclin D1: E and H - Control and Emodin alone (expression not detectable), F - DMBA alone (over-expressed), G - DMBA + Emodin (down-regulated).
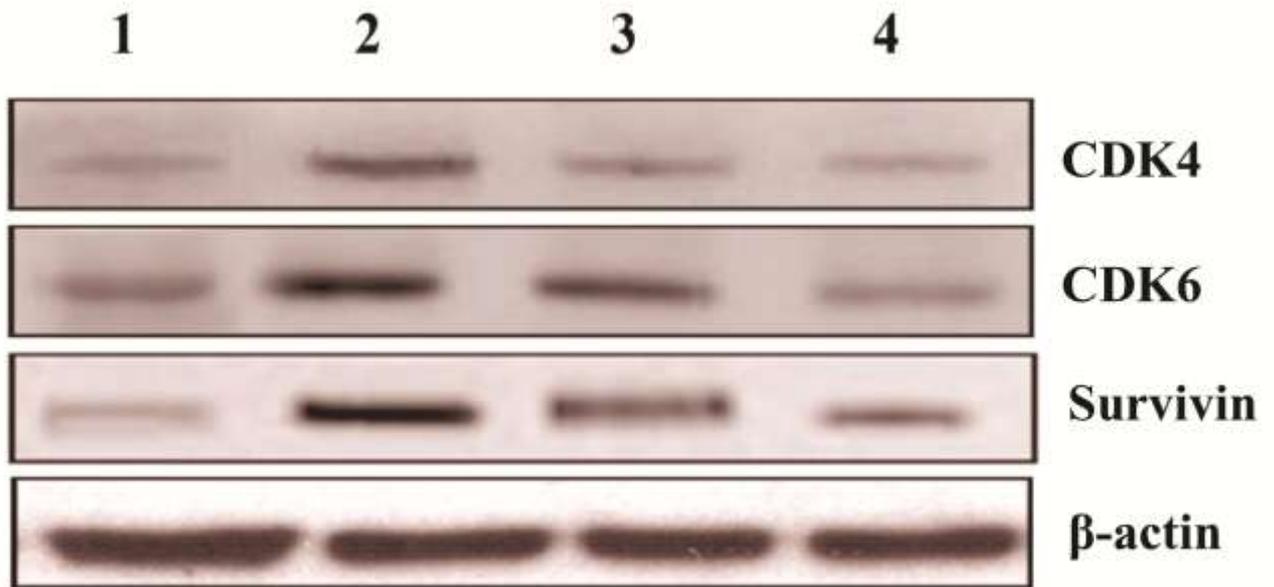
Figure 2: Expression pattern of buccal mucosa CDK4, CDK6 and Survivin in the experimental hamsters.
Lane 1: Control, Lane 2: DMBA alone, Lane 3: DMBA + Emodin, Lane 4: Emodin alone.
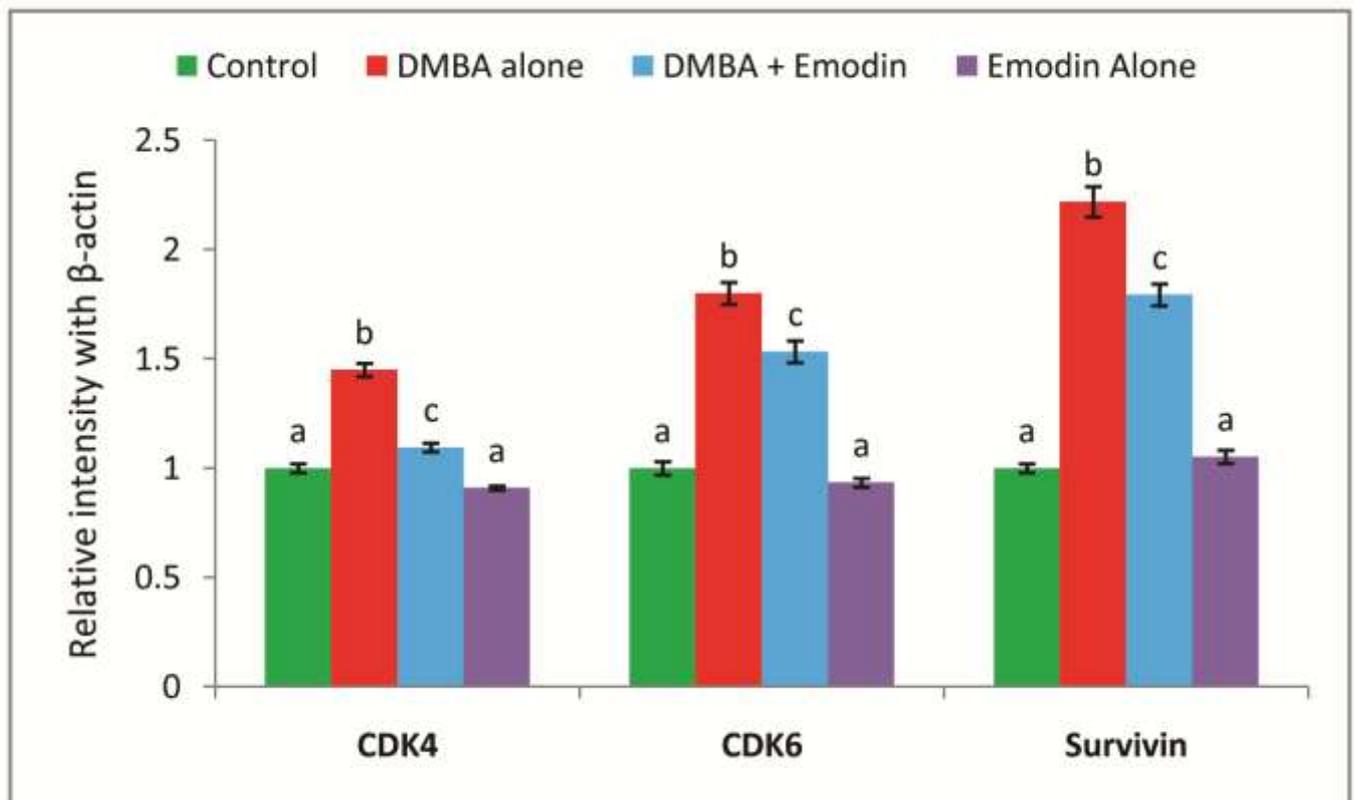
Figure 3: Densitometric analysis of protein expression after normalization to β-actin in the buccal mucosa of experimental hamsters

Data presented are the mean ± SD (n=10). Common superscripts between two groups - not significant. Different superscripts between two groups - significant $p<0.05$.

Discussion

Biomarkers play a key and critical function in determining the tumor stage as well as to plan the treatment options. Tumor progression occurs due to deregulation in the molecular pathways that regulate cellular differentiation and proliferation (Zhao and Ramaswamy, 2014). The cross talking between various genes either directly or indirectly could lead to tumorigenesis. A spectrum of biomarkers has been utilized to assess the disease status in various diseases, including cancer (Brookes and Shi, 2014). The researchers utilized the molecular biomarkers that has a vital function in the carcinogenic process to explore the anti-cell proliferative efficacy of natural products or synthetic entities (Ovadje et al., 2015). The present study explores the anti-cell proliferative potential of Emodin by analysing the expression pattern of cell proliferation markers in DMBA induced oral carcinogenesis.

Imbalance in cell proliferation is one of the characteristic features of carcinogenesis. Abnormal expression of cyclin D1 was shown in various types of malignancies, including oral carcinoma (Lee et al., 2012). Cyclin D1 expression was associated with the tumor staging, lymphnode metastasis, grading and tumor size/thickness (Sarkar et al., 2015). Cyclin D1 over-expression has been correlated with the shortening of G1 phase. Though the cyclin D1 expression was seen in 73% of oral cancer cases, its expression was not connected with the survival outcome of the patients treated with preoperative chemoradiation (Basnaker, 2014). Zhang et al., (2012) reported that cyclin D1 status could be utilized used as a prognostic marker for tumor recurrence. Angadi and Krishna pillai (2007) showed a positive cyclin D1 expression in 75% of oral carcinoma cases. Zhang et al., (2012) showed a cyclin D1 abnormal expression in 65% of laryngeal squamous cell carcinoma patients. Saawarn et al., (2012) showed a direct correlation between the cyclin D1 expression and cell differentiation in oral cancer. Huang et al., (2012) pointed out that cyclin D1 over-expression was associated with the poor clinical outcome in Taiwanese oral cancer patients. Several studies have thus pointed out the cyclin D1 as a significant molecular target for the suppression of oral carcinogenesis.

Cyclin D1, CDK4 and CDK6 play a vital function in the G1 phase progression as well as in the G1→S transition (Pestell, 2013). Cyclin D1 triggers the gene transcription necessary for G1→S phase transition via binding and activating CDK4, CDK6 (Neganova and Lako, 2008). In this study, abnormal expression of cyclin D1, CDK4 and CDK6 was observed in the tumor tissues of all the hamsters treated with DMBA alone. Emodin administration orally to DMBA treated hamsters lowered the expression of cyclin D1, CDK4 and CDK6. Sui et al., (2014) demonstrated the inhibitory effect of Emodin on breast cancer cell proliferation. They suggested that Emodin inhibited breast cancer cell proliferation via down-regulation of cyclin D1 expression. Wang et al., (2015) suggested that Emodin exhibited antitumor potential against gynecological cancer cells via down-regulating cyclin D and cyclin E. Zhang et al., (2015) reported that Emodin suppressed the cell growth and proliferation of oral cancer cells by down-regulating the cell proliferation markers such as CDK2 and cyclin E. The present results indicate that Emodin might have significantly suppressed the cell proliferation of oral carcinoma cells in the buccal mucosa, as evidenced by down-regulation of CDK4, CDK6 and cyclin D1 in the DMBA+Emodin treated hamsters.

PCNA has been highlighted as a putative prognostic cell proliferative marker in various malignancies including oral carcinoma. Abnormal PCNA expression was shown in oral carcinogenesis (Al-Azzawi, 2014; Oliveira and Ribeiro-Silva, 2011). Survivin has a key role in the process of mitosis and cell proliferation (Xie et al., 2015). Survivin expression was abnormal in various cancers, including oral carcinoma (Jaiswal et al., 2015). A positive correlation has been reported between abnormal expression of survivin and patients with poor prognosis (Mobahat et al., 2014). It has been reported that survivin expression was predominant in the proliferating basal cell layer of the oral tumor cell mass (Xie et al., 2015). Kim et al., (2011) pointed out the parallel link between the survivin expression and tumor lymphnode metastasis in oral carcinogenesis. Su et al., (2010) suggested that survivin mRNA expression can be utilized as an independent prognostic factor for oral carcinoma patients. The present study observed PCNA and survivin over-expression in the buccal mucosa of hamsters treated with DMBA alone. Emodin administration to hamsters treated with DMBA down-regulated PCNA and survivin expression, which suggest that Emodin significantly inhibited the cell proliferation during DMBA induced oral carcinoma. The present study thus pointed out the modulatory effect of Emodin on cell proliferation markers during DMBA induced oral carcinogenesis. The possible mechanism of Emodin efficacy on abnormal cell proliferation is given in the figure 4.
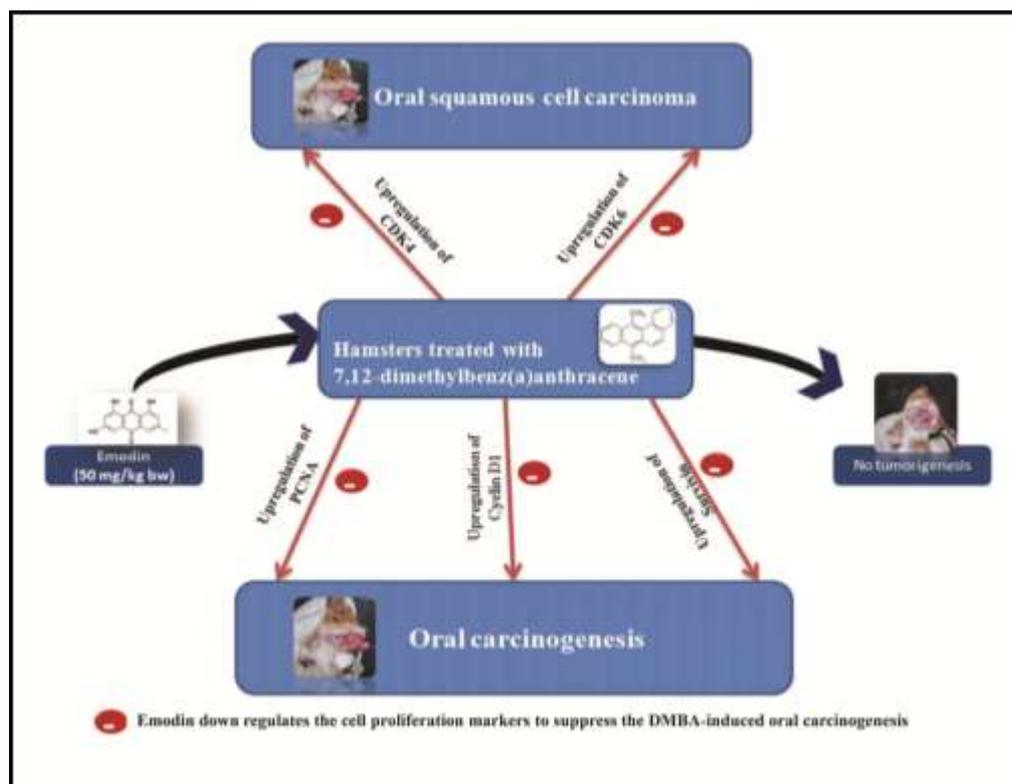
Figure 4: The possible mechanism of Emodin efficacy on abnormal cell proliferation

Conclusion

The present research findings, explores the protective role of Emodin on abnormal cell proliferation in DMBA induced oral carcinoma in golden Syrian hamsters. The anti-cell proliferative potential of Emodin is owing to its modulating consequence on cell-cycle markers towards tumor suppression during DMBA induced oral carcinogenesis in golden Syrian hamsters.

Acknowledgements

References

1. Al-Azzawi, L.M. (2014). Immunohistochemical Analysis of PCNA and P53 Proteins in Oral Lichen Planus, Oral Dysplasia and Normal Oral Mucosa. Diyala J Med. **6**(4):41–47.
2. Angadi, P.V. and Krishnapillai, R. (2007). Cyclin D1 expression in oral squamous cell carcinoma and verrucous carcinoma: correlation with histological differentiation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. **103**(3):30–35.
3. Arvindekar, A., More, T., Payghan, P.V., Laddha, K. and Ghoshal, N. (2015). Evaluation of anti-diabetic and alpha glucosidase inhibitory action of anthraquinones from Rheum emodi. Food Funct. 6(8):2693-2700.
4. Asghar, U., Witkiewicz, A.K., Turner, N.C. and Knudsen, E.S. (2015). The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Dis. **14**(2): 130–146.
5. Basnaker, M. (2014). Cyclin D1 gene expression in oral mucosa of tobacco chewers – An immunohistochemical study. J Clin Diagn Res. **8**(5):70–75.
6. Bertoli, C., Skotheim, J.M. and de Bruin, R.A.M. (2013). Control of cell cycle transcription during G1 and S phases. Nat Rev Mol Cell Biol. **14**(8):518–528.
7. Bhadauria, M. (2010). Dose-dependent hepatoprotective effect of emodin against acetaminophen-induced acute damage in rats. Exp Toxicol Pathol. **62**(6):627-635.

8. Brookes, E. and Shi, Y. (2014). Diverse epigenetic mechanisms of human disease. Annu Rev Genet. 48(1):237-268.
9. Chen, G., Zhang, J., Zhang, H., Xiao, Y., Kao, X., Liu, Y. and Liu, Z. (2015). Anti-inflammatory effect of emodin on lipopolysaccharide-induced keratitis in Wistar rats. Int J Clin Exp Med. **8**(8):12382–12389.
10. Chen, X., Duan, N., Zhang, C. and Zhang, W. (2016). Survivin and tumorigenesis: molecular mechanisms and therapeutic strategies. J Cancer. **7**(3):314–323.
11. Diamant, N., Hendel, A., Vered, I., Carell, T., Reißner, T., de Wind, N. and Livneh, Z. (2012). DNA damage bypass operates in the S and G2 phases of the cell cycle and exhibits differential mutagenicity. Nucleic Acids Res. **40**(1):170–180.
12. Gondak, R.O., da Silva-Jorge, R., Jorge, J., Lopes, M. A. and Vargas, P.A. (2012). Oral pigmented lesions: Clinicopathologic features and review of the literature. Med Oral Patol Oral Cir Bucal. **17**(6):e919–e924.
13. Hebbar, P.B., Sheshaprasad, R., Gurudath, S., Pai, A. and Sujatha, D. (2014). Oral submucous fibrosis in India: Are we progressing?? Indian J Cancer. 51(3):222-226.
14. Huang, S.F., Cheng, S.D., Chuang, W.Y., Chen, I.H., Liao, C.T., Wang, H.M. and Hsieh, L.L. (2012). Cyclin D1 over-expression and poor clinical outcomes in Taiwanese oral cavity squamous cell carcinoma. World J Surg Oncol. **10**(1):40.
15. Jaiswal, P.K., Goel, A. and Mittal, R.D. (2015). Survivin: A molecular biomarker in cancer. Indian J Medl Res, **141**(4):389–397.
16. Karthikeyan, S., Srinivasan, R., Wani, S.A. and Manoharan, S. (2013). Chemopreventive potential of chrysin in 7,12-dimethylbenz(a)anthracene-induced hamster buccal pouch carcinogenesis. Int J Nutr Pharmacol Neurol Dis. **3**(4):46-53.
17. Khalil, M.I., Ibrahim, M.M., El-Gaaly, G.A. and Sultan, A.S. (2015). *Trigonella foenum* (Fenugreek) induced apoptosis in hepatocellular carcinoma cell line, hepg2, mediated by up-regulation of p53 and proliferating cell nuclear antigen. Biomed Res Int. **2015**:e914645.
18. Kim, G.Y., Lim, S.J. and Kim, Y.W. (2011). Expression of HuR, COX-2, and survivin in lung cancers; Cytoplasmic HuR stabilizes cyclooxygenase-2 in squamous cell carcinomas.Mod Pathol. **24**(10):1336-1347.
19. Kwon, M.J. (2013). Emerging Roles of Claudins in Human Cancer. Int J Mol Sci. **14**(9):18148–18180.
20. Lee, S.S., Tsai C.H., Tsai, L.L., Chou, M.C., Chou, M.Y. and Chang, Y.C. (2012).β-catenin expression in areca quid chewing-associated oral squamous cell carcinomas and up-regulated by arecoline in human oral epithelial cells. J Formos Med Assoc. **111**(4):194-200.
21. Lim, S. and Kaldis, P. (2013). Cdks, cyclins and CKIs: roles beyond cell cycle regulation. development. **140**(15):3079-3093.
22. Maaly, A.B., Madeeha, A., Maryam, K., James, A.R. and Heba, M.E. (2015). Tobacco Consumption and Oral, Pharyngeal and Lung Cancers. Open Cancer J. **8**:1-11.
23. Manoharan, S., Karthikeyan, S., Essa, M.M., Manimaran, A. and Selvasundram, R. (2016). An overview of oral carcinogenesis. Int J Nutr Pharmacol Neurol Dis. **6**:51-62.
24. Manoharan, S., Rajasekaran, D., Prabhakar, M. M., Karthikeyan, S. and Manimaran, A. (2015). Modulating effect of *Enicostemma littorale* on the expression pattern of apoptotic, cell proliferative, inflammatory and angiogenic markers during 7, 12-dimethylbenz (a) anthracene induced hamster buccal pouch carcinogenesis. Toxicol Int, **22**(1):130–140.
25. Mobahat, M., Narendran, A. and Riabowol, K. (2014). Survivin as a preferential target for cancer therapy. Int J Mol Sci. **15**(2):2494–2516.
26. Neganova, I. and Lako, M. (2008). G1 to S phase cell cycle transition in somatic and embryonic stem cells. J Anatomy. **213**(1):30-44.
27. Oliveira, L.R. and Ribeiro-Silva, A. (2011). Prognostic significance of immunohistochemical biomarkers in oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 40(3):298-307.
28. Ovadje, P., Roma, A., Steckle, M., Nicoletti, L., Arnason, J.T. and Pandey, S. (2015). Advances in the research and development of natural health products as main stream cancer therapeutics. Evid Based Complement Alternat Med. **2015**:751348.
29. Pestell, R.G. (2013). New roles of cyclin D1. AM J Pathol. **183**(1):3–9.
30. Saawarn, S., Astekar, M., Saawarn, N., Dhakar, N. and Gomateshwar Sagari, S. (2012). Cyclin D1 Expression and Its Correlation with Histopathological Differentiation in Oral Squamous Cell Carcinoma. Sci World J. **2012**: e978327.
31. Sarkar, S., Kanoi, A., Bain, J., Gayen, R. and Das, K.N. (2015). Correlation between cyclin D1 expression and standard clinicopathological variables in invasive breast cancer in Eastern India. South Asian J Cancer. **4**(4):155–159.
32. Siddiqi, K., Shah, S., Abbas, S. M., Vidyasagaran, A., Jawad, M., Dogar, O. and Sheikh, A. (2015). Global burden of disease due to smokeless tobacco consumption in adults: analysis of data from 113 countries. BMC Medicine. **13**(3):191-194.

33. Siegel, R.L., Miller, K.D. and Jemal, A. (2016) Cancer statistics, 2016. CA Cancer J Clin. **66**(1):7-30.

34. Strzalka, W. and Ziemienowicz, A. (2011). Proliferating cell nuclear antigen (PCNA): a key factor in DNA replication and cell cycle regulation. Ann Botany. **107**(7):1127–1140.

35. Su, L., Wang, Y., Xiao, M., Lin Y. and Yu, L. (2010). Up-regulation of survivin in oral squamous cell carcinoma correlates with poor prognosis and chemoresistance. Oral Surg Oral Medicine, Oral Pathology, Oral Radiology. 110(4):484-491.

36. Sui, J.Q., Xie, K.P., Zou, W. and Xie, M.J. (2014). Emodin inhibits breast cancer cell proliferation through the ERα-MAPK/Akt-cyclin D1/Bcl-2 signaling pathway. Asian Pac J Cancer Prev. **15**(15):6247-6251.

37. Tanaka, T. and Ishigamori, R. (2011). Understanding Carcinogenesis for Fighting Oral Cancer. J Oncol. 2011:e603740.

38. Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet-Tieulent, J. and Jemal, A. (2015). Global cancer statistics, 2012. CA Cancer J Clin. 65(2):87-108.

39. Velez, A.M.A. and Howard, M.S. (2015). Tumor-suppressor genes, cell cycle regulatory checkpoints, and the skin. NAJ Medical Sciences. **7**(5):176–188.

40. Wang, W., Sun, Y., Li, X., Li, H., Chen, Y., Tian, Y., Yi., J. and Wang, J. (2011). Emodin potentiates the anticancer effect of cisplatin on gallbladder cancer cells through the generation of reactive oxygen species and the inhibition of survivin expression. Oncol Rep. **26**:1143-1148.

41. Wang, Y., Yu, H., Zhang, J., Ge, X., Gao, J., Zhang, Y. and Lou, G. (2015) Anti-tumor effect of emodin on gynecological cancer cells. Cell Oncol (Dordr). **38**(5):353-363.

42. Xie, S., Xu, H., Shan, X., Liu, B., Wang, K. and Cai, Z. (2015). clinicopathological and prognostic significance of survivin expression in patients with oral squamous cell carcinoma: evidence from a meta-analysis. PLoS ONE **10**(2): e0116517.

43. Xiong, H.R., Luo, J., Hou, W., Xiao, H. and Yang, Z.Q. (2011). The effect of emodin, an anthraquinone derivative extracted from the roots of *Rheum tanguticum*, against herpes simplex virus in vitro and in vivo. J Ethnopharmacol. 133(2):718-723.

44. Zhang, K., Jiao, K., Zhu, Y., Wu, F., Li, J. and Yu, Z. (2015). Effect of emodin on proliferation and cell cycle of human oral squamous carcinoma Tca8113 cells *in vitro*. Nan Fang Yi Ke Da Xue Xue Bao. **35**(5):665-670.

45. Zhang, L.Q., Jiang F, Xu L, Wang J, Bai JL, and Yin, R. (2012). The role of cyclin D1 expression and patient's survival in non-small-cell lung cancer: a systematic review with meta-analysis. Clin Lung Cancer.**13**(1):188–195.

46. Zhao, M. and Ramaswamy, B. (2014). Mechanisms and therapeutic advances in the management of endocrine-resistant breast cancer. World J Clin Oncol. **5**(3): 248–262.