Mammalian target of rapamycin and head and neck squamous cell carcinoma

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Abstract

Head and neck squamous cell carcinoma (HNSCC), a significant cause of cancer deaths worldwide, has multiple stepwise malignant evolutions. Mammalian target of rapamycin (mTOR) plays a critical role in tumor development, invasion, metastasis and angiogenesis that impact local recurrence and survival. mTOR can also act as a biomarker for personalized adjuvant therapy. In in vivo and in vitro studies, mTOR inhibitor suppresses tumor growth and sensitizes HNSCC to radiation, cytotoxic agents and epidermoid growth factor receptor inhibitors. We have reviewed the pathogenesis of HNSCC, mTOR pathway, mTOR inhibitor and the role of mTOR in HNSCC.

Review

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide and accounts for approximately 650,000 new diagnoses and 350,000 cancer deaths every year [1]. Smoking and alcohol are the most well known carcinogens of HNSCC [2]. In some areas of Asia, chewing betel quid, a psychoactive substance that always contains areca nut, betel leaf and calcium hydroxide, is a distinct risk factor that exerts a synergistic effect with smoking and alcohol consumption for oral and laryngeal cancer [3,4]. In addition, the continuation of smoking and alcohol consumption after initial diagnosis of HNSCC increases the risk for secondary primary cancer [5]. Human papillomavirus (HPV), predominantly type 16, infection inducing genomic instability is another mechanism for tumorigenesis in the oropharynx that is distinct from the role of smoking or alcohol [6].

Surgery and radiotherapy are the main modality of HNSCC treatment [7]. Chemotherapy, acting as a radiosensitizer, increases survival in locally advanced disease [8,9]. To treat early disease, surgery is preferred. Radiotherapy is an alternative method for organ preservation for laryngeal cancer [10,11]. In unresectable settings, concurrent cisplatin chemoradiotherapy that provides better disease free survival and overall survival than radiotherapy alone is the standard of care [9]. Surgery-treated, advanced patients with high risk factors can also obtain benefit of local and regional control and progression free survival by adding concurrent chemotherapy to postoperative radiotherapy [12]. Overall, the incorporation of concurrent chemoradiotherapy to management of HNSCC absolutely increases survival rate by 6.5% at year-five [13]. Recently, cetuximab, an epidermal growth factor receptor-specific monoclonal antibody, plus radiation were shown to improve survival rate as compared to radiation treatment alone [14]. However, a retrospect study suggests the duration of progression free survival and overall survival is shorter in patient receiving cetuximab plus radiation than those with cisplatin plus radiation [13]. Multi-modality treatment or targeted therapy containing management does not significantly improve overall survival.

HNSCC has a complex mechanism of carcinogenesis that involves multiple genetic abnormalities, stepwise evolution and signaling pathway alternation [7,15-18]. Alternations of p53, p16 and cyclin D1 (CCND1) result in limitless growth of tumor cells [4,19-22]. Change of epidermal growth factor receptor (EGFR), c-MET, phosphatidylinositol 3-kinase, catalytic, alpha polypeptide (PIK3CA), Ras-mitogen-activate protein kinase (Ras-MAPK), phosphatase and tensin homolog (PTEN) and transforming growth factor-beta (TGF-beta) are essential to affect growth factor signaling that impact cell proliferation, apoptosis and survival [23-28]. High expression of nuclear factor Kappa B (NF-Kappa B), surviving and B cell lymphoma -2 (Bcl-2) are positively associated with poor survival [29-31].

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Target of rapamycin (TOR) pathway

Mammalian TOR (mTOR), a protein kinase encoded by FK506 binding protein 12-rapamycin associated protein 1 (FRAP1) gene [32], is an important downstream target signal of PI3K pathway. (Figure 1) [33]. The protein contains an 12-kDa FK506-binding protein (FKBP12), rapamycin binding domain, Huntington Elongation Factor 3 PR65/ATOR (HEAT) motifs, FK506 binding protein 12-rapamycin associate protein (FRAP1)-ataxia telangiectasia mutated (ATM)-transformation transcription domain-associated protein (FAT) and FAT C terminus (FATC) domain. In terms of structure and function, mTOR consists of two distinct complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [34,35]. mTOR, regulatory-associated protein of mTOR (Raptor) and G-protein-subunit-like protein form mTORC1, a nutrition-sensitive complex. mTORC1 is sensitive to rapamycin, control cell growth and is a key factor of the mTOR pathway [34-38]. mTORC2, a complex containing mTOR, G-protein-subunit-like protein and mAVO3, regulates the actin cytoskeleton and is insensitive to rapamycin [39]. As an important target kinase of the PI3K pathway, mTOR responds to multiple stimuli including: nutrients, insulin, oxygen, growth factor, ATP, Ras homologue enriched in brain (RHEB) and tobacco components [33,38,40-44]. However, mTOR is negatively regulated by complex of tuberin and hamartin [45]. Through the activation of two downstream targets p70S6K and 4EBP1, mTOR functions on translation, cell growth, protein synthesis, cell size and angiogenesis [46-48]. Activated p70S6K stimulates 5-terminal oligopyrimidine (5'TOG) translation to regulate ribosome biogenesis [49]. Phosphorylated 4EBP1 dissociates with eIF4E. The free eIF4E, an oncoprotein, promotes cap-dependent translation with subsequent regulation of c-myc, cyclin D1, ornithinedecarboxylase, basic fibroblast growth factor (b-FGF), vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) to affect cell survival, tumorigenesis and transformation, angiogenesis, invasion and metastasis [41,50-54]. In addition, mTOR-enhanced expression of HIF-1a protein, HIF-1 transcriptional activity, and VEGF protein are the key regulators in angiogenesis [55]. Apoptosis signal-regulating kinase 1 (ASK1)-modulated apoptosis can be inhibited by mTOR-induced overexpression of protein phosphatase 5 (PP5) [56].

mTOR inhibitor

Many compounds, including rapamycin, rapalog and adenosine-5'-triphosphate (ATP)-competitive inhibitor, have been shown to block the activation of the mTOR pathway [57]. Rapamycin, an antifungal agent, [58], binds to the FKBP12-rapamycin (FRB) domain of mTORC1 to interrupt downstream activation [59]. Poor water solubility, absorption, limited bio-availability, hepatic first-pass effect and drug interaction account for interpatient variability that requires therapeutic drug monitoring for the complex pharmacokinetic behaviors [60]. Although rapamycin is a promisingly cytostatic anticancer agent in the National Cancer Institute’s screening program, [35], those pharmacologic characteristics limit the practical application [61,62]. In order to improve the pharmacokinetic features of rapamycin, a chemical modification at C-40-0 can develop three new rapalog including everolimus, temsirolimus and ridaforolimus that share the same mechanisms of action. They not only exert anti-cancer activity but also act as a sensitizer to radiotherapy and chemotherapy. Frequent adverse events such as fatigue, mucositis, rash, anorexia, diarrhea, nausea, thrombocytopenia, leukopenia, anemia, hyperglycemia, hyperlipidemia and hypercholesterolemia

Figure 1 Mammalian target of rapamycin is a key regulator in development and progression of cancer. Mammalian target of rapamycin responds to stimuli of growth factor, insulin, tobacco components, nutrients, hypoxia, ATP and RHEB to activate P70S6 and inhibit 4EBP1 and PPS with subsequent dysregulation of apoptosis, cell survival, cell transformation, tumorigenesis, angiogenesis, invasion and metastasis. PI3K, phosphatidylinositol 3-kinase; ATP, adenosine triphosphate; RHEB, ras homologue enriched in brain; mTORC1, mammalian target of rapamycin complex 1; PP5, protein phosphatase 5; ASK1, apoptosis-signal-regulating kinase 1; p70S6, ribosomal p70 S6; EIF4E, eukaryotic translation initiation factor 4E; 4E-BP1, EIF4E-binding protein 1.
are limited and manageable [63]. Everolimus (RAD001) is an oral rapalog, and has better oral absorption and bioavailability profiles than compared to those of rapamycin [64,65]. It also shows sustained inhibition of S6K1 activity at the dose of ≥ 20 mg weekly and ≥ 5 mg daily [66]. Temsirolimus is a prodrug converted into rapamycin after intravenous infusion. It exerts evidence of activity over a dose range between 15 and 300 mg/m², and has the dose-limiting toxicity from thrombocytopenia [67]. Ridaforolimus is a non-prodrug rapalog, and has dose limiting toxicity from mouth sore at 28 mg/d and maximal tolerable dose of 18.5 mg/d [68]. In a study of skin biopsy specimens, ridaforolimus significantly suppressed the expression of 4EBP1, S6 and extracellular signal-regulated kinase (ERK) [69].

**mTOR pathway and HNSCC**

HNSCC amplifies eukaryotic translation initiation factor 4E (eIF4E) gene and overexpresses eIF4E protein [70]. The tumor itself, the surgical margins, and even the histologically normal epithelium in the margins were all shown to overexpress eIF4E. The strong association of elevated activity of eIF4E with high expression of mTOR downstream signals transduction (phospho-4E binding protein 1, S6, phospho-mTOR) and elevated level of AKT expression suggests the activation of AKT/mTOR pathway in the margin. High expression of phospho-P70S6 and AKT in the margin indicates that the activity of AKT/mTOR cascade is higher in tumor margin than in the tumor itself [71]. There is a significant correlation between degree of expression of eIF4E in the margin and grading of the dysplasia (P = .006) [72]. eIF4E is essential in the malignant progression of HNSCC [70]. Interestingly, higher activity of eIF4E in the tumor margin, even those free of microscopic tumor, is an independent predictor of local recurrence while histological grading of dysplasia failed to predict prognosis [73]. Nathan et al examined tumor samples from 65 patients. All biopsies expressed elevated levels of eIF4E. The intra-tumor activity of eIF4E was not different between the eIF4E-positive and -negative margin groups. Thirty-six patients (55%) with microscopic tumor-free margins had eIF4E expression in the basal cell layer of the margin. After a median follow-up of 17 months, local-regional recurrence developed in 20 patients (56%) with eIF4E-positive margins. In contrast, two patients (6.9%) with absence of eIF4E expression had local recurrence after median follow-up of 14.5 months. Histologically tumor-free margin with high expression of eIF4E has a seven-fold risk of local failure. The median disease free duration is significantly shorter in the eIF4E positive margin group (eIF4E positive versus negative, 11 months versus 14 months; log-rank test, P = .0001). The prediction of recurrence by expression of eIF4E in HNSCC margin is independent from tumor size, nodal status, stage, histologic grade, tumor site, eIF4E levels in the tumor, and with the degree of dysplasia in the margins [72]. Also, the level of p-S6 expression significantly increases with the malignant progression of the tumor [74]. In addition, irradiation, an important treatment of HNSCC, promotes the expression of mTOR and AKT in HNSCC cells [75]. High expression of AKT sensitizes mTOR inhibition through down-regulation of cyclin D1 and c-myc [76,77]. Activation of AKT/mTOR pathway plays a key role in tumorigenesis and survival rate of HNSCC patients [71]. The eIF4E is a potential maker to define the molecular-free surgical margin, and is a promising predictor of survival [72,73].

**mTOR inhibitor and HNSCC**

Temsirolimus blocks the activation of mTOR pathway in HNSCC cell line to reduce the expression of S6 and 4E-BP1 with subsequently suppressed expression of FGF and VEGF that inhibited cell growth in vitro. In a xenograft study, the 4E-BP1 activity of tumor cells and peripheral blood mononuclear cells (PBMC) is also reduced by mTOR inhibition [78,79]. Rapamycin treatment increased nuclei apoptosis in tumor in situ TUNEL assay, and reduced neovascularization [74]. To mimic patients with histologically tumor-free margin with high expression of eIF4E, the tumor cells in the culture medium were introduced into the dorsal flap of nude mice with pipettes to establish a minimal residual disease model (MRD). Measuring the tumor formation at day 21 after xenograft, the treatment group had a significantly longer median tumor free duration (treatment versus control group, 18 days versus 7 days; P < 0.0001). The tumor size of treatment group was significantly smaller than those of the control group (P < 0.0001). In the “survival study” mTOR inhibition delayed the time to develop tumors with the volumes of at least 200 m³ in the MRD model (P < 0.0001). Twenty-one percent of the treated mice were free of tumors 30 days after the discontinuation of the treatment. As expected, temsirolimus treatment significantly reduced photon emission on bioluminescence imaging. The reduction increased with the continuation of the treatment. The result of the MRD model suggests that the prolonged mTOR inhibition may have clinical benefits in the adjuvant setting for patients with eIF4E positive margin [78]. mTOR inhibitor is a potential agent in HNSCC treatment. Phosphorylated mTOR, eIF4E, and high expression of AKT may be potential biomarkers in order to select the candidate HNSCC patients for mTOR inhibitor-based adjuvant therapy [71,77,80].

Everolimus enhances DNA-damage agent-induced apoptosis in tumor cells. It overcomes cisplatin resistance in small cell and non-small cell lung cancer cell
lines, [81,82], and sensitizes cancer cells to radiation by arresting cells in G2M phase [79,83,84]. In an in vivo study, temsirolimus was shown to block signal transduction of mTOR pathway to decrease VEGF production, but failed to sensitize HNSCC to radiation by clonogenic assay. In a study with cisplatin-sensitive Fa-Du and cisplatin-resistant SCC-40 xenografts receiving 3-week treatment with temsirolimus or cisplatin plus radiation, temsirolimus alone treatment, even at low doses, significantly blocked the tumor growth in both xenografts. The combination of temsirolimus with radiation (XRT) more significantly promoted radiation-induced tumor reduction ($P < 0.05$; temsirolimus plus XRT versus. temsirolimus or XRT alone) than compared to the combination of cisplatin with XRT alone in both cisplatin-sensitive and resistant cell lines ($P < 0.05$). Addition of cisplatin to the temsirolimus and XRT treatment failed to increase the therapeutic effect. The sensitization effect by temsirolimus is evidenced by the following: the reduced phosphorylation of 4EBP1, S6 and Bad; the increased number of radiation-related poly(ADP-ribose) polymerases (PARPs) cleavage; the increased rate of nuclei apoptosis; and the reduction of tumor vascularity by diminishing VEGF production. The median survival time was 49 days for the temsirolimus plus XRT treatment group, 38 days for the cisplatin plus XRT treatment group and 27 days for the control group for the SCC-40 cell lines. Treatment with temsirolimus alone or with the combination of XRT can significantly increase the survival rate of SCC-40 xenograft as compared to the control group ($P < 0.05$). mTOR inhibitor is a promising radio-sensitizer in HNSCC treatment [75]. Although EGFR is an important target of therapy, [85]. HNSCC poorly responds to or is refractory to anti-EGFR treatment. In HNSCC cell lines Detroit 562, erlotinib blocks the activation of MAPK and suppresses the expression of AKT and p70. Temsirolimus alone failed to affect AKT and MAPK. The MAPK was completely blocked by the combination treatment while the activity of AKT was significantly inhibited. In an in vivo study, the combination therapy, erlotinib alone therapy, and the temsirolimus alone therapy obtained growth rates that was 18%, 34% and 13% of the rate of growth of the control group, respectively. Seven days after the treatment, the expression of pMAPK, Ki-67 and phospho-p70 were significantly reduced. mTOR inhibition suppressed tumor growth of EGFR-resistant cell lines and exerted an additive effect with the combination of the EGFR inhibitor [86].

7 Few HNSCC patients were enrolled into a phase 1 study to investigate the safety of an mTOR inhibitor based combination therapy. A patient with HNSCC T4N3M1 with lung metastasis with failed responses to docetaxel, cisplatin and zalutumumab partially responded to temsirolimus and metformine [87]. One oropharyngeal cancer patient obtained stable disease

| Drug             | Study phase | Treatment design                          | Disease status                      | Clinicaltrial.gov identifier |
|------------------|-------------|-------------------------------------------|-------------------------------------|------------------------------|
| Rapamycin        | I/II        | Neoadjuvant with 21-day rapamycin followed by surgery | Stage III or IVA, resectable        | NCT01195922                  |
| Temsirolimus     | II          | Temsirolimus with or without cetuximab    | Recurrent or metastasis             | NCT01256385                  |
|                  | II          | Temsirolimus alone                        | Recurrent or metastasis             | NCT01172769                  |
|                  | I/II        | Temsirolimus + Weekly paclitaxel + carboplatin   | Recurrent or metastasis             | NCT01016769                  |
|                  | I/II        | Temsirolimus, cisplatin, and cetuximab    | Recurrent or metastasis             | NCT01015664                  |
|                  | II          | Temsirolimus and erlotinib               | Platinum-refractory or -ineligible, advanced disease | NCT01009203                  |
| Everolimus       | I           | Everolimus, weekly cisplatin and XRT      | Locally advanced                    | NCT01058408                  |
|                  | I           | Induction with everolimus, docetaxel, and cisplatin   | Locally advanced                    | NCT00935961                  |
|                  | I           | Everolimus, weekly cisplatin and XRT      | Locally advanced                    | NCT00858663                  |
|                  | I           | Everolimus, cisplatin and XRT             | Locally advanced                    | NCT01057277                  |
|                  | I/II        | Induction everolimus paclitaxel, and cisplatin   | Locally advanced                    | NCT01133678                  |
|                  | II, randomized | Adjuvant everolimus Vs placebo       | Locally advanced disease after definite local treatment | NCT01111058                  |
|                  | I/IIB       | Everolimus, carboplatin, and cetuximab    | Recurrent or metastasis             | NCT01283334                  |
|                  | I/IIB       | Everolimus, cetuximab and cisplatin        | Recurrent or metastasis             | NCT01009346                  |
|                  | II          | Everolimus                               | Refractory, recurrent or metastasis | NCT01051791                  |
|                  | II          | Everolimus, erlotinib                    | Recurrent                           | NCT00942734                  |
| Ridaforolimus    | I           | Ridaforolimus, cetuximab                 | Advanced                            | NCT01212627                  |
after more than 6 cycles of treatments with everolimus and weekly cisplatin. No change of expression of p21, p53 or p-AKT was found on a biopsy specimen from pretreatment and day 21 on treatment [88].

Many studies have been initiated to elucidate the role of mTOR inhibitor in the treatment of HNSCC (Table 1). National Institutes of Dental and Craniofacial Research initiated a pilot study to investigate the efficacy and molecular change of neoadjuvant 3-week treatment of rapamycin in resectable HNSCC patients. Molecular study of the specimens obtained from tumor biopsies during the period of treatment provides further information for clinical response to rapamycin (clinicaltrial.gov identifier: NCT01195922). One future randomized phase II trial of everolimus versus placebo as an adjuvant therapy in patients with locally advanced HNSCC (NCT01111058) will evaluate the benefit of long-term mTOR inhibition in patients with eIF4E positive margin [78]. Some trials will test the safety at different dosages and determine the optimal dose of mTOR inhibitor in combination with radiation or cytotoxic agents.

**Conclusion**

mTOR plays an important role in the complex carcinogenesis of HNSCC, predicts survival, and may be a potential biomarker to identify candidate patients for mTOR inhibition-based adjuvant therapy. Many preclinical experiments suggest that the mTOR blockade has anti-tumor activity, displays radio- or chemo-sensitization, and overcomes the EGFR resistance. Further clinical trial results may provide more information about the role of mTOR in future studies and management of HNSCC.

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**Authors’ contributions**

YY designed the paper. YY, YML and CK wrote the paper. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Parfin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005, 55:74-108.
2. Vines P, Alavanja M, Buffle P, Fontham E, Franceschi S, Gao YT, Gupta PC, Hackshaw A, Matos E, Samet J, et al. Tobacco and cancer: recent epidemiological evidence. J Natl Cancer Inst 2004, 96:99-105.
3. Wen CP, Tsai MK, Chung WS, Hsu HL, Chang YC, Chan HT, Chiang PH, Cheng TY, Tsai SP. Cancer risks from betel quid chewing beyond oral cancer: a multiple-site carcinogen when acting with smoking. Cancer Causes Control 2010, 21:1427-1435.
4. Mack TM. The new pan-asian paan problem. The Lancet 2001, 357:1638-1639.
5. Do KA, Johnsen MM, Doherty DA, Lee JJ, Wu YF, Dong Q, Hong WK, Khuri FR, Fu KK, Spitz MR. Second primary tumors in patients with upper aerodigestive tract cancers: joint effects of smoking and alcohol (United States). Cancer Causes Control 2003, 14:131-138.
6. D’Souza G, Kreemer AR, Viscidi R, Pavia T, Falkhy C, Koch WM, Westra WH, Gillison ML: Case-Control Study of Human Papillomaviruses and Oropharyngeal Cancer. New England Journal of Medicine 2007, 356:1944-1956.
7. Haddad RI, Shin DM. Recent advances in head and neck cancers. N Engl J Med 2008, 359:1143-1154.
8. Cohen EE, Lingen MW, Vokes EE. The expanding role of systemic therapy in head and neck cancer. J Clin Oncol 2004, 22:1743-1752.
9. Adelstein DJ, Li Y, Adams GL, Wagner H Jr, Kish JA, Ensley JF, Schuller DE, Forastiere AA: An intergroup phase III comparison of standard radiation therapy and two schedules of concurrent chemoradiation therapy in patients with unresectable squamous cell head and neck cancer. J Clin Oncol 2003, 21:92-98.
10. Argiris A, Karamouzis MV, Raben D, Fentis RL: Head and neck cancer. The Lancet 2008, 371:1695-1709.
11. Jones AS, Fish B, Fenton JE, Husband DJ: The treatment of early laryngeal cancers (T1-T2 NO): surgery or irradiation? Head & Neck 2004, 26:127-135.
12. Berrnick J, Domenge C, Oszainh M, Manuszewka K, Lefebvre B, Greiner RH, Grait J, Maigont P, Rolland F, Bolla M, et al: Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med 2004, 350:1945-1952.
13. Pignon JP, Le Maitre A, Maillard E, Boursi J: Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. Radiother Oncol 2009, 92:4-14.
14. Bonnier JA, Harati PM, Grait J, Azamia N, Shin DM, Cohen RB, Jones CU, Sur R, Raben D, Jassem J, et al: Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006, 354:567-578.
15. Wang X, Fan M, Chen X, Wang S, Alsharif MJ, Wang L, Liu L, Deng H: Intratumor genomic heterogeneity correlates with histological grade of advanced oral squamous cell carcinoma. Oral Oncol 2006, 42:740-744.
16. Leemans CR, Braakhuis BJ, Braakhoff RH: The molecular biology of head and neck cancer. Nat Rev Cancer 2011, 11:9-22.
17. Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 2000, 100:57-70.
18. Matta A, Ralhan R: Overview of current and future biologically based targeted therapies in head and neck squamous cell carcinoma. Head Neck Oncol 2009, 1:6.
19. Somers KD, Merck MA, Lopez NE, Incogno LS, Schechter GL, Casey G: Frequent p53 Mutations in Head and Neck Cancer. Cancer Research 1992, 52:5997-6000.
20. Boyle JO, Hakim J, Koch W, van der Riet P, Hruban RH, Roa RA, Correo R, Carson DA, Ridge JA, Goodrow TL: The incidence of p53 mutations increases with progression of head and neck cancer. Cancer Res 1993, 53:4477-4480.
21. Callender T, El-Naggar AK, Lee MS, Frankenthaler R, Luna MA, Batsakis JG: PIK3CA Mutations in Head and Neck Squamous Cell Carcinoma. Cancer Research 2003, 64:5996-6000.
22. Khuri FR, Fu KK, Spitz MR: Second primary tumors in patients with unresectable squamous cell head and neck cancer. New England Journal of Medicine 1994, 331:152-158.
23. Zhang SY, Klein-Szanto AJ, Sauter ER, Shafarek M, Mutsuga N, Nobori T, Carson DA, Ridge JA, Goodrow TL: Higher frequency of alterations in the p16/CDKN2 gene in squamous cell carcinoma cell lines than in primary tumors of the head and neck. Cancer Res 1994, 54:3050-3053.
24. Grandis JR, Tweardy DJ: Elevated Levels of Transforming Growth Factor α and Epidermal Growth Factor Receptor Messenger RNA Are Early Markers of Carcinogenesis in Head and Neck Cancer. Cancer Research 1993, 53:3579-3584.
25. Marshall DD, Kornberg LJ: Overexpression of scatter factor and its receptor (c-met) in oral squamous cell carcinoma. Laryngoscope 1998, 108:1419-1417.
26. Kozaki K, Imoto I, Pimkhakham A, Hasegawa S, Tsuda H, Omura K, Inazawa J: PIK3CA mutation is an oncogenic aberration at advanced stages of oral squamous cell carcinoma. Cancer Sci 2006, 97:1351-1358.
27. Qiu W, Schönleben F, Li X, Ho DJ, Close LG, Manolidis S, Bennett BP, Su GH: PIK3CA Mutations in Head and Neck Squamous Cell Carcinoma. Clinical Cancer Research 2006, 12:1441-1446.
27. Okami K, Wu L, Riggins G, Cairns P, Goggin M, Eronen E, Halachmi N, Ahrendt SA, Reid AL, Hilgiers W, et al. Analysis of PTEN/MMAC1 Alterations in Aerodigestive Tract Tumors. Cancer Research 1998, 58:509-511.

28. Wang Z, Song H, Evans JA, Lang JC, Schaller DE, Weihehorst CM. Mutation and downregulation of the transforming growth factor beta type II receptor gene in primary squamous cell carcinomas of the head and neck. Carcinogenesis 1997, 18:2285-2290.

29. Duffey DC, Chen Z, Dong G, Ondrey FG, Wolf JS, Brown K, Siebenlist U, Van Waes C. Expression of a Dominant-Negative Mutant Inhibitor xbd ± of Nuclear Factor-κB in Human Head and Neck Squamous Cell Carcinoma Inhibits Survival, Proinflammatory Cytokine Expression, and Tumor Growth in Vivo. Cancer Research 1999, 59:3468-3474.

30. Lin-CY, Hung HC, Kuo R-C, Chiang CP, Kuo MY-P. Survivin expression predicts poorer prognosis in patients with areca quid chewing-related oral squamous cell carcinoma in Taiwan. Oral Oncology 2005, 41:654-655.

31. Gallo O, Boddi V, Calzolari A, Simonetti L, Trovati M, Bianchi S. Bcl-2 protein expression correlates with recurrence and survival in early stage head and neck cancer treated by radiotherapy. Clin Cancer Res 1996, 2:261-267.

32. Moore PA, Rosen CA, Carter KC. Assignment of the Human FKBP12-Rapamycin-Associated Protein (FRAP) Gene to Chromosome 1p36 by Fluorescencencen Situhybridization. Genomics 1996, 33:331-332.

33. Carott PH, Brown CL, Johns AD, Roth RA, Lawrence PC. Evidence of insulin-stimulated phosphorylation and activation of the mammalian target of rapamycin mediated by a protein kinase B signaling pathway. Proceedings of the National Academy of Sciences of the United States of America 1998, 95:7772-7777.

34. Loewith R, Jacinto E, Wulfschlegler S, Lorberg A, Crespo JL, Bonenfant D, Oppolzer W, Jenne P, Hall MN. Two TOR Complexes, Only One of Which Is Sensitive to Rapamycin. Science 2002, 298:403-408.

35. Byrom SJ, Houghton PJ. The TOR pathway: a target for cancer therapy. Nat Rev Cancer 2004, 4:335-348.

36. Hara K, Mauro Y, Leng X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J. Yorezynski Y, Raptor, a Binding Partner of Target of Rapamycin (TOR), Mediates TOR Action. Cell 2002, 110:177-189.

37. Kim D-H, Sarbassov DD, Ali SM, Latch K, Rurt K, Gurna R,开端mTOR Complex Activity. J Biol Chem 2002, 277:141-150.

38. Kim DH, Sarbassov DD, Ali SM, Latch K, Rurt K, Gurna R,开端mTOR Complex Activity. Journal of Biological Chemistry 2002, 277:141-150.

39. Zhang B, Cao H, Rao GH. 15(S)-Hydroxyeicosatetraenoic Acid Induces Angiogenesis via Activation of PI3K-Akt-mTOR-S6K1 Signaling. Cancer Research 2005, 65:7283-7291.

40. Zheng Z-Z, Yellatru CR, Neele I, Rao GH. 5(S)-Hydroxyeicosatetraenoic Acid Stimulates DNA Synthesis in Human Microvascular Endothelial Cells via Activation of Jak/STAT and Phosphatidylinositol 3-Kinase/Akt Signaling. Leading to Induction of Expression of Basic Fibroblast Growth Factor 2. Journal of Biological Chemistry 2002, 277:4121-4129.

41. Jeffries HH, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G. Rapamycin suppresses S [prime]TOP mRNA translation through inhibition of p70s6k. EMBO J 1997, 16:3693-3704.

42. De Benedetti A, Graff JR. elf-4E expression and its role in malignancies and metastases. Oncogene 2000, 23:3189-3199.

43. Jones RM, Branda J, Johnston KA, Polyemini M, Gadd M, Rustgi A, Callanan L, Schmidt EV. An essential E box in the promoter of the gene encoding the mTOR mRNA cap-binding protein (eukaryotic initiation factor 4E) is a target for activation by c-myc. Mol Cell Biol 1996, 16:4734-4746.

44. Rosenwald I, Zalans-Karazas A, Sonenberg N, Schmidt E. Elevated levels of cyclin D1 protein in response to increased expression of eukaryotic initiation factor 4E. Mol Cell Biol 1993, 13:7358-7363.

45. Kevil CG, De Benedetti A, Payne DK, Coe LL, Lauzoux FS, Alexander JS. Translational regulation of vascular permeability factor by eukaryotic initiation factor 4E: implications for tumor angiogenesis. Int J Cancer 1996, 65:785-790.

46. Ji, Yang M. et al. Regulation of Matrix Metalloproteinase-9 (MMP-9) by Translational Efficiency in Murine Prostate Carcinoma Cells. Cancer Research 2002, 62:1910-1914.

47. Zhong H, Chiles K, Feldner D, Laughner E, Hanahan C, Georgescu MM, Simons JW, Semenza GL. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 2000, 60:1541-1545.

48. Huang S, Shu L, Easton J, Harwood FC, Germsac GS, Ichijo H, Houghton PJ. Inhibition of Mammalian Target of Rapamycin Activates Apoptosis Signal-regulating Kinase 1 Signaling by Suppressing Protein Phosphate 5 Activity. Journal of Biological Chemistry 2004, 279:36490-36496.

49. Liu Q, Thoreen C, Wang J, Sabatini D, Gray NS. mTOR Mediated Anti-Cancer Drug Discovery. Drug Discov Today Ther Strateg 2009, 6:47-55.

50. Vealinsa C, Kadivnekk S, Seghal SN. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. J Antibiot (Tokyo) 1975, 28:721-726.

51. Brown EJ, Albers MW, Bum Shin T, Ichikawa K, Keith CT, Lane WS, Schriefler SL. A mammalian protein target by G1-arresting rapamycin-receptor complex. Nature 1994, 369:756-758.

52. Nappi RL, Taylor PJ. From beach to bedside: history of the development of sirolimus. Ther Drug Monit 2009, 31:539-546.

53. Huang S, Byrom SJ, Houghton PJ. Rapamycin: mechanism of action and cellular resistance. Cancer Biol Ther 2003, 2:222-232.

54. Boni JP, Hug B, Leister C, Sonnichsen D. Intravenous Temsirolimus in Cancer Patients: Clinical Pharmacology and Dosing Considerations. Seminars in Oncology 2009, 36:518-525.

55. Kirchner GI, Meier-Wiedenbach I, Manns MP. Everolimus. Cancer Patients: Clinical Pharmacology and Dosing Considerations. Seminars in Oncology 2009, 36:518-525.

56. Huang S, Byrom SJ, Houghton PJ. Rapamycin: mechanism of action and cellular resistance. Cancer Biol Ther 2003, 2:222-232.

57. Brown EJ, Albers MW, Bum Shin T, Ichikawa K, Keith CT, Lane WS, Schriefler SL. A mammalian protein target by G1-arresting rapamycin-receptor complex. Nature 1994, 369:756-758.

58. Nappi RL, Taylor PJ. From beach to bedside: history of the development of sirolimus. Ther Drug Monit 2009, 31:539-546.

59. Huang S, Byrom SJ, Houghton PJ. Rapamycin: mechanism of action and cellular resistance. Cancer Biol Ther 2003, 2:222-232.

60. Boni JP, Hug B, Leister C, Sonnichsen D. Intravenous Temsirolimus in Cancer Patients: Clinical Pharmacology and Dosing Considerations. Seminars in Oncology 2009, 36:518-525.

61. Kirchner GI, Meier-Wiedenbach I, Manns MP. Everolimus. Cancer Patients: Clinical Pharmacology and Dosing Considerations. Seminars in Oncology 2009, 36:518-525.
9. MacKenzie M, Ernst S, Johnson C, Winquist E. A phase I study of temsirolimus and metformin in advanced solid tumours. Investigational New Drugs. 2010;1:6.
10. Fury MG, Sherman EJ, Wu N, Hague S, Lisa DM, Carlson D, Pfister DG. Phase I study of everolimus (E) plus low-dose weekly cisplatin (C) for patients with advanced solid tumors. ASCO Meeting Abstracts. 2010, 28:e13013.

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