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Potential Role of EGF and EGFR in Tongue Coating Formation

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ABSTRACT

Tongue coating, as a sensitive index of the physiological and pathological changes of human organs, is an important basis for the Traditional Chinese Medicine (TCM) syndrome differentiation and treatment. Mechanism of the formation and change of tongue coating is an important scientific problem. In recent years, researches indicated that in addition to apoptosis related genes, epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) also contribute to tongue coating formation. In this paper, we summarized recent studies on the potential role of EGF and EGFR in regulating the formation of tongue coating to provide some reference for the further investigations on tongue coating formation mechanisms.

Key words: Tongue coating, epidermal growth factor, epidermal growth factor receptor, review
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INTRODUCTION

In Traditional Chinese Medicine (TCM), tongue diagnosis has been used as a non-invasive and valuable diagnostic tool for thousands of years. TCM treats tongue appearance as an outer manifestation of the status of the human body. TCM practitioners differentiate conditions of individual patients according to the TCM Syndromes ("ZHENG" in Chinese), which are used as a holistic summary of the patient's status and are determined mainly by tongue coating color and tongue texture of the patient in addition to other symptoms. Tongue appearance includes tongue coating and tongue body. The tongue coating refers to fur-like substances covering the surface of tongue body, caused by "Wei-Qi". Clinical researches have reported that tongue body changes slowly while tongue coating changes rapidly and obviously in the development of many diseases. Tongue coating, in TCM, is believed to reflect the severity of disease and determine TCM syndrome. Different tongue image with different clinical significance, corresponding to different methods of treatment based on syndrome differentiation. The relationships between tongue diagnosis and diseases and TCM syndrome have been explored extensively. However, the biological bases of different tongue coating appearance are still poorly understood and systematic investigations are scarce. So, revealing the potential mechanism of tongue coating is full of importance.

Biological Functions of EGF and EGFR

EGF is a kind of single chain polypeptide containing 53 amino acid residues, which was found in 1962 by Cohen in the mouse submandibular gland[1]. In this study, EGF was found to promote the newborn mice early opening eyes and teething and make the weight loss and delay hair growth. The main biological function of EGF is to regulate the proliferation and migration of epithelial cells, endothelial cells, fibroblasts and stromal cells, and so on. EGF was also found to promote the normal cell malignant transformation and tumor progression by increasing tumor cells proliferation, angiogenesis, invasion and metastasis[2]. Epidermal growth factor receptor (EGFR) is the only receptor of EGF, which is widely distributed in the epithelial cell membrane in addition to the vascular tissue. EGFR is a transmembrane receptor tyrosine kinase which is activated by several ligands leading to the activation of various signaling pathways regulating key processes of cellular functions, including proliferation, differentiation and survival during development, tissue homeostasis, and tumorigenesis.

EGFR is known as ErbB1 or HER-1, belonging to the family of receptor tyrosine kinases (RTK)[3]. Structurally, EGFR is composed of an extracellular domain, where its ligands bind to, followed by a transmembrane domain and an intracellular domain, where the tyrosine kinase domain and the carboxy-terminal tail containing key tyrosine residues are located[4]. Canonical ligands that can bind EGFR include epidermal growth factor (EGF), transforming growth factor α (TGF-α), amphiregulin (AR), epiregulin (EPR), betacellulin (BTC), and heparin-binding EGF (HB-EGF)[5].

Upon activation through the binding of one of its ligands, EGFR can form homo- or hetero-dimers with other EGFR family members, resulting in receptor activation.

Following activation of the intrinsic kinase domain, several proteins containing Src-homology 2 domains (SH2) such as growth factor receptor-bound protein 2 (Grb2), SHC-transforming protein (SHC), and phospholipase C γ (PLC γ) can bind to the phosphorylated tyrosine residues within the EGFR and activate complex downstream signaling cascades that drives multiple cellular responses, including changes in gene expression, cytoskeletal rearrangement, apoptosis inhibition, and increased cell proliferation. The main activated downstream signaling pathways are the Ras-Raf-MEK-ERK1/2 and the signal-transducer and activator of transcription (STAT) 3 and 5 pathways controlling proliferation and differentiation and the phosphatidylinositol-3-kinase (PI3K)-Akt-mechanistic target of rapamycin (mTOR) pathway controlling biological functions[6-7].

EGF and EGFR in Clinical

Under normal circumstances, epidermal growth factor receptor tyrosine kinase (EGFR-TK) activity can be accurately control. The gene mutation or abnormal expression activity of EGFR-TK will be continued to improve, causing abnormal several intracellular signal transduction pathway, which leads to a variety of diseases[8]. EGF and EGFR are expressed in many tissues and facilitate excessive/uncontrolled cell growth and tumorigenesis via multiple signal transduction pathways and participate in cellular proliferation and differentiation. Abnormal expression and mutation of EGF and EGFR play an important and well-recognized role in oncogenesis. For instance, the over expression of EGF or EGFR have been causally implicated in breast cancers[9], non-small-cell lung cancer[10], ovarian cancer[11], Hepatocellular carcinoma[12], etc. As the over expression of EGF and EGFR was correlated to a poorer clinical prognosis and predicts a bad response to chemotherapy in a number of cancer forms, EGFR has been especially focused upon as predictive biomarker and target molecules for cancer treatments. A variety of small molecule kinase inhibitors targeting EGFR and monoclonal antibodies targeting EGFR have been developed and some of them have already been used for treatment of lung cancer and breast cancer, etc.

Besides the above-mentioned functions, the EGFR signaling path has been suggested to play a key role in liver regeneration following acute and chronic liver damage, as well as in cirrhosis and hepatocellular carcinoma (HCC)[13]. EGF and EGFR has been demonstrated to accelerate the skin healing in trauma, surgery and burns[14], the gastrointestinal ulcers healing and cornea injury remodeling[15], and postpone skin aging by promoting the hyaluronic acid and glycoprotein secretion in cells.

Different Expression Levels of EGF and EGFR in the Patients with Different Tongue Coating

EGF and EGFR are important regulating factors of the cells differentiation proliferation, apoptosis. From recently studies, people gradually realized that formation of tongue coating essentially is the process of tunica mucosa linguae epithelial cells constantly differentiation, proliferation and apoptosis. When the process is relatively balanced, tongue coating presents normal thin white fur. But any part of this process is affected may cause the change of tongue coating[16]. Tongue coating has been revealed to related to the EGF and EGFR expression.

Zhen et al[17] enrolled 425 volunteers including tumor and non-tumor patients and healthy control to investigate the relationship between different tongue coatings and the contents of EGF in tongue coating and saliva by Radioimmunoassay (RIA). The results indicated that tongue coating of tumor patients was generally thicker than that of non-tumor patients and healthy controls. EGF in saliva was higher in either tumor or non-tumor patients with thick tongue coating, than that of patients with thin fur or health control. The level of EGF in the saliva is closely related to whether the tongue coating is thick or thin and whether the coating is peeled or not. The level of EGF in saliva of cancer patients is significantly higher than non-cancer patients.

Xiao et al[18] studied the relationship between EGF levels and changes of tongue coating in heroin addicts after detoxification. In this study, the levels of EGF in saliva and tongue coating of heroin addicts after detoxification for 15–30 days and healthy people were measured. Results indicated that proportion of thick tongue coating in addicts was higher than that in the control group, and the EGF levels in the addicts with thick tongue coating was significantly reduced compared to the addicts with thin tongue coating group and control group.

Chen et al[19] detected the EFG levels in saliva of patients with chronic superficial gastritis, chronic atrophic gastritis and healthy control by radioimmunoassay. Results demonstrated that compared to healthy controls the levels of EGF in saliva and EGFR expression levels of patients with chronic gastritis of different tongue images were increased and enhanced, and they also increased and enhanced in patients with thick tongue coating and yellow tongue coating compared with patients with thin tongue coating and white tongue coating.

In order to investigate the relationship between serum EGF and tongue coating of patients with gastric carcinoma, Dong et al[20] measured serum EGF levels of patients with gastric carcinoma and healthy controls by ELISA. Results showed that thick tongue coating accounted for the greatest proportion in cases with gastric carcinoma, while thin tongue coating was more popular in the healthy control. Serum EGF levels in patients were higher than that in healthy. In patient with yellow thin tongue coating, serum EGF presented the highest levels, then followed by those with yellow thick and exfoliative tongue coating. In healthy control. Serum EGF levels were the highest in healthy cases with yellow thick coating, followed by those with yellow thin coating and exfoliative coating. Proportion of thick tongue coating in patients was higher than that in the healthy. Increase in

Serum EGF levels was observed in the patients with gastric carcinoma.

Liu et al[21] measured the EGF contents of patients with chronic hepatitis B, patients with other digestive diseases, patient with other system diseases and healthy people by radioimmunoassay. EGFR of tongue epithelial cells was detected by immune-histochemical method. Results showed that the contents of EGF in saliva and serum as well as the expression levels of EGFR in tongue epithelium of abnormal tongue coating group all increased compared with those of healthy people. Especially in the group of white thick and yellow greasy tongue coating, EGF and EGFR increased significantly. The serum contents of HBV-DNA of white thick and yellow greasy tongue coating group of Chronic Hepatitis B (CHB) patients was highest, compared with the thin white coating group and the difference was significant. Therefore, they proposed that the forming of abnormal tongue coating of CHB patients was related to the multiplication of tongue epithelium that accelerated by EGF in saliva.

In conclusion, no matter in patients with cancer or other diseases, the proportion of people with thick tongue coating is much higher than that in normal subjects. The levels of EGF in serum, saliva or tongue coating of patients with thick tongue coating was higher, which was thought to be closely related to the formation and thickening of tongue coating. In addition, the changes of EGF or EGFR contents in saliva were consistent to that in serum, which provided a possibility of detecting the contents of EGF and EGFR in saliva and tongue coating samples instead of blood samples. EGF contents differentiated from patients with different tongue coating. In both of patients and healthy controls, the expression levels of EGF and EGFR were higher in people with thick and yellow tongue coating, followed by those with thin and white tongue coating and exfoliative tongue coating. But this trend did not completely or always coincide, such as in heroin addicts the h-EGF levels in patients with thin fur was higher than that of patients with thick tongue coating (Table 1). Manifestations above laid the foundation for us to study the effect of EGF and EGFR in tongue coating formation.

Table 1. Expression levels of EGF or EGFR in different tongue coating.

Disease categories	Sample source	Methods	Test content	Expression levels
Tumor 17	Saliva	Radioimmunoassay	h-EGF	Thick coating > thin coating > no coating; Yellow coating > white coating
Heroin addicts after detoxification 18	Saliva	Radioimmunoassay	h-EGF	Thick coating < thin coating
Chronic gastritis 19	Saliva	Radioimmunoassay	h-EGF	Thick coating > thin coating > normal thin tongue; Yellow coating > white coating > normal white tongue
	Tongue coating	Immunohistochemical	EGFR in tongue coating exfoliated cells	Red tongue > pink tongue > Light white tongue > normal pink tongue
Gastric carcinoma 20	Serum	ELISA	Serum EGF	Yellow thin coating > yellow thick coating > no coating
Chronic hepatitis B 21	Saliva and Serum	Immunohistochemical, TUNEL, FQ-PCR	Saliva and Serum EGF	Yellow greasy coating > white thick coating > no coating and less coating > white thin coating > white yellow coating > control

The Role of EGF and EGFR in Tongue Coating Formation

1 EGF promotes the expression of EGFR

Signaling routing mediated by EGFR initiates after ligand and receptor binding, and then homo-or heterodimers formed, crosslinked phosphorylation happens afterwards. Intracellular region is activated and signal transduction is initiated next gives full play to cells in a variety of biological effects[22].

Zhou et al[23] reported that EGF can affect the formation of tongue coating through EGF-R initiates. In their study EGF was injected to observe the growth of tongue epithelium of mice and EGF-R expression, so as to investigate into the molecular mechanism of EGF in affecting the formation of

tongue coating. Ordinary grade ICR male mice were divided into experimental and control groups. Mice in the experimental group were injected subcutaneously with 0.2 mL EGF (Containing EGF200 μg, Sigma crude product) and in control group, the mice were injected with 0.2 mL saline. After 2 h, 24 h and 48 h of administration respectively, tongue tissue was harvested and immediately fixed in 10% formaldehyde. After HE staining and the EGFR SABC immune histochemical staining, the number of the tongue epithelial cells and the expression of EGFR were observed. Results showed that with the extension of injection time, tongue epithelial basal layer cells increased, the staining changing depth and cell division phase increased, the tongue coating was gradually thickened. At the same time, the expression of EGFR was also increased with the increased of time.

Zhan et al[24] observed the relationship between tongue epithelial cell growth and EGFR expression in mice after oral dropping EGF, and discovered that exogenous EGF promoted the mice tongue mucosa EGFR expression along with tongue coating thickened.

Xu et al[25] applied flow cytometry to investigate the effects of EGF on cell cycle and EG F-R expression of esophagus carcinoma cell line Eca-109 and explore molecular mechanism of influencing tongue fur of the tumor patients by EGF. Results revealed that EGF can significantly promote the expression of EGFR in Eca109 cell membrane and the cell proliferation activity is greatly enhanced.

Zhen et al[26] investigated correlation between the levels of EGF and tongue coating formation in tongue cancer patients, total RNA of tongue epithelial tissue was prepared and detected by cDNA micro array respectively, and the EGFR mRNA expression was measured by real-time PCR. Results demonstrated that the gradations from high to low according to the contents of EGFR mRNA was yellow thick tongue coating, white thin tongue coating, yellow thin tongue coating, non-tongue coating, white thick tongue coating. And each of them was higher than control group.

Tong et al[27] using immune histochemical S-P method to investigate patients with tongue squamous cell carcinoma of different tongue mucosa EGFR expression and revealed that EGFR expression levels from high to low was: thin white fur, thick white fur, thin yellow fur, thick yellow fur, and in normal control and stripping tongue coating without expression, which was contrary to the above results.

Zhang et al[28] enrolled 387 cases of gastric cancer patients and 392 healthy people participate in their study. By using the polymerase chain reaction-ligase specific inspection technology (PCR-LDR) they found that in patients with different tongue images the EGFR gene polymorphism was different. rsl05017 and rs2293347 significantly influenced changes of tongue coating color, while rsl7337023, rsl050171 and rs2293347 significantly influenced changes of tongue nature.

The research results above together showed that the expression levels of EGFR in different tongue epithelial cells were significantly different. EGF may make the EGFR expression increased by autocrine or paracrine mechanism, which acts on the EGFR signal transduction pathways, involved in regulating the dynamic balance of the tongue coating formation and changes.

2 EGF and EGFR promote the apoptosis related gene expression

Cell apoptosis is an active death process controlled by genes, such as p53, Bax, Fas, c-myc, caspases-3, Ras, Bcl-2, P35. Studies showed that tongue coating formation and dorsal lingual mucosa epithelial cell proliferation, differentiation, migration and shedding is closely related to the apoptosis associated gene expression, which is one of the important mechanism to the change of the thickness of tongue coating[29-30]. When the proliferation, differentiation and apoptosis of tongue epithelial cells are in a relatively balanced state, the tongue is characterized by thin tongue coating. When the cell proliferation was dominant, apoptosis and differentiation were at a disadvantage, the tongue epithelial cells increased and the tongue is characterized by thick tongue coating[31].

Liu Xin et al[21] found that, in chronic hepatitis B patients with yellow greasy tongue coating and white thick tongue coating, the serum EGF content was significantly higher than that of the normal control group and CHB patients with other kinds tongue coating, while the tongue epithelial cell apoptosis index (AI) is the lowest. It was indicated that EGF correlated to apoptosis of tongue epithelial cells.

Xu et al[32] investigated the effects of EGF on the expressions of Fas and c-myc protein expression in Tca-8113 cell membrane and found that after administration of EGF for 12 h, cells in 5 ng/mL and 10 ng/ml EGF groups, the expression levels of Fas and c-myc were significantly higher than that in control group. It suggested that EGF promoted the expression of apoptosis related genes Fas and the proliferation and differentiation related genes c-myc in the epithelial cells of the tongue, and the promoting effect on c-myc was stronger. This study demonstrated that EGF probably promoted the tongue epithelial cell apoptosis and proliferation by increasing Fas and c-myc expression.

3 EGF influences the formation of tongue by affecting adhesion molecule expression

Molecule adhesion (AM) is a class of membrane surface glycoprotein that mediates adhesion between cells, cells and extracellular matrix including integrin family, mucin like family, selectin family, immunoglobulin superfamily and E-cadherin in five categories. AM plays an important role in cell proliferation and differentiation, inflammation, immune response, coagulation, thrombosis and so on. CD29 is a member of the integrin family, which can mediate the adhesion of cells to the extracellular matrix, so that the cells can be attached to form a whole[33]. CD54 and CD106 belong to the immunoglobulin superfamily, and the main function is to mediate cell adhesion.

Xu et al[34] studied effects of EGF on tongue Tca-8113 cell lines by measuring the changes of CD29, CD54, CD106 expression levels in the cell membrane. Results showed that

with the increase of time and the increase of EGF concentration, CD29 expression significantly increased. The expression of CD54 in each dose group was significantly increased after EGF effecting eight hours. After 16 hours, the expression of CD54 in each dose group was still higher than that in the control group, but not statistically, indicating that CD54 had no continuous increasing effect on the expression of EGF. In this experiment, the change of expression of CD64 was not obvious. The results showed that EGF probably regulated tongue coating formation by promoting the expression of CD29 and CD54 and mediating adhesion in the tongue private membrane epithelial cell.

E-cadherin (E-cad) is another important member of adhesion molecules[35], which participates in intercellular adhesion and maintains cell polarity and structural integrity. Studies have indicated that many malignant tumors are associated with decreased expression of E-cad or loss of function, so E-cad is considered to be a tumor suppressor. However, there are also some studies showed that the abnormal expression of E-cad is closely related to the occurrence and metastasis of the tumor. It is suggested that E-cad can not only inhibit the cancer, but also promote the cancer. Zhan et al[36] used gene chip and real-time RT PCR technology to detect the expression levels of E-cad mRNA in different tongue coating. Microarray analysis showed that the expression level of E-cad mRNA in different tongue was remarkably different. Quantitative RT-PCR analysis showed that the expression of E-cad mRNA from high to low was as follows: yellow thick tongue coating, thin yellow tongue coating, stripping tongue coating, white thick tongue coating, thin white tongue coating, fetal thin white tongue coating. From this phenomenon they come to that E-cad gene expression may be an important downstream event of EGF and EGF-R pathway involved in the formation of tongue coating.

4 EGF through the promotion of keratinized epithelial cells to promote thickening of tongue coating

Tongue mucosa epithelium belongs to stratified squamous epithelium tissue. In normal conditions, the metabolism of tongue epithelial cells is in a state of relative balance, most of which manifests as the incomplete keratinization cells, keratinized cells and keratinized cells were in the minority, and there were almost no middle layer cells. In pathological conditions, exfoliated cells of tongue morphology will change accordingly.

Lu et al[37] tried to explore the effects of EGF on esophageal cancer cell line Eca-109 cells. They found that in medium without EGF, more than 90% of the Eca-109 was mild hyperkeratosis and highly keratinized cells only account for 0.19%. In medium containing EGF, keratinocyte increased in EGF-concentration dependent manner. Especially when the EGF concentration was 1.0 g/mL the number of slight keratinization cells reduced and highly keratinized cell increased significantly showed the strongest promoting cornification effects on Eca-109 cells. So they speculated that EGF promote the keratinized epithelial cells may be another important mechanism for the formation of tongue. But the relevant reports are still few and not yet to be verified.

Problems and Prospects

Tongue diagnosis is a useful method to examine the physiological functions and pathological conditions of the human body in TCM. In the long history of traditional clinical practice in China, TCM practitioners have typically classified patients of the same disease into sub-groups as different Syndromes from a holistic perspective on patients' overall status. Many features are used in the discrimination of Syndromes, but tongue coating appearance is the major factor. TCM believes that white tongue coating mainly represents exterior syndrome and cold syndrome, while yellow tongue coating mainly represent interior syndrome and heat syndrome. The thickness of tongue coating can reflect the balance between Evil Factors and Health Principle of body as well as depth of the pathogenic qi, which plays an important role in reflecting the occurrence, development, and prognosis of the disease and the clinical syndrome differentiation and treatment. Therefore, revealing the mechanism of tongue coating formation and change is full of great significance, which cannot only provide objective basis for tongue diagnosis of TCM theory, but also helps to open up a new prospect of clinical application of tongue diagnosis.

Morphological study showed that tongue coating is mainly composed of comprises filiform papilla, large amount of desquamated epithelial cells released from the oral or tongue mucosa, seeping leukocyte, blood metabolites, bacteria, saliva, food debris and other common compositions. The proliferation and differentiation degree of filiform papillae mainly determines the thickness of tongue coating. Exogenous EGF or the abnormal increased EGF in saliva, blood and tissue in diseased bodies may act on EGFR in tongue epithelial cells and at the same time induces cells expressing more EGFR leading to an increase in the use of EGF. Subsequently, increased EGF and EGFR induce the expression of apoptosis related genes increased, prompting tongue epithelial cell apoptosis. On the other hand, EGF and EGFR also can strongly promote the expression of gene associated with the proliferation such as c-myc significantly promote the lingual mucosa epithelial cell proliferation. At the same time, they can induce the expression of adhesion molecules, which can promote cell adhesion and aggregation, and control the proliferation of lingual epithelial cells. So as to promote the formation of pathological thick tongue fur and fur color deepening (Table 2).

It has been understood that human microbiome is related to human health status and many diseases. Some researches have revealed that tongue coating appearance may reflect characteristics of the tongue microbiome and the bacteria structure was associated with the formation and change of tongue coating. Li Shao et al[38] recruited 19 gastritis patients

Table 2. Potential role of EGF and EGFR in tongue coating formation and change.

Possible mechanisms	Cell, Tissue	Methods	Major findings	References No.
Expression of EGFR	Mouse tongue epithelial cells	HE staining, Immumohistochemical staining	After hypodermic injection of EGF, the tongue coating was gradually thickened and the expression levels of EGFR was also increased with the increased of time.	23
	Mouse tongue epithelial cells	HE staining, Immunohistochemical staining	Exogenous EGF promoted the mice tongue mucosa EGFR expression along with tongue coating thickened.	24
	Esophageal cancer cell Eca 109	Flow cytometry	EGF can significantly promote the expression of EGFR in Eca109 cell membrane and the cell proliferation activity is greatly enhanced.	25
	Tongue mucosa from tongue squamous cell carcinoma patients	Gene chip, FQ-PCR	The expression level of EGFR mRNA of different tongue have significant difference.	26
	Blood cells	PCR-LDR	The EGFR gene polymorphism can significantly influenced changes of tongue coating color and tongue nature.	28
Apoptosis related gene expression	Tongue coating smear	Immunohistochemical, TUNEL, FQ-PCR	The serum EGF content in yellow greasy tongue coating was highest, while the tongue epithelial cell apoptosis index (AI) was the lowest.	21
	Tongue Tca-8113 cell lines	Flow cytometry	EGF promoted the expression of apoptosis related genes Fas and the proliferation and differentiation related genes c-myc in the epithelial cells of the tongue.	32
Adhesion molecule expression	Tongue Tca-8113 cell lines	Flow cytometry	EGF probably regulated tongue coating formation by promoting the expression of CD29 and CD54 and mediating adhesion in the tongue private membrane epithelial cell.	34
	Mucous membrane of tongue	Gene chip, RT-PCR	E-cad mRNA expression level in different tongue coating have significant difference.	36
Epithelial cells keratization	Esophageal cancer cell Eca 109	Fluorescence labeling, Flow cytometry	Exogenous EGF can promote the tongue coating epithelial cells keratinized.	37

with a typical white-greasy or yellow-dense tongue coating corresponding to TCM Cold or Hot Syndrome respectively, as well as eight healthy volunteers into a study. In the study their tongue coating microbiome was profiled by Illumina paired-end, double-barcode 16S rRNA sequencing. The results showed that approximately 3.7 million V6 tags for each sample were obtained. They identified 123 and 258 species-level OTUs that were enriched in patients with white-greasy or yellow-dense tongue coating corresponding to Cold/Hot Syndromes, respectively. The results demonstrated a potential and important connection between tongue coating microbiome and traditional tongue diagnosis. Han Shuwen et al[39] used the DS01-B tongue diagnostic information acquisition system to photograph and analyze tongue and tongue coating, and next-generation sequencing technology was used to determine the V2-V4 hypervariable regions of 16S rDNA to investigate the micro-biome on tongue coating. Results showed that, comparing with healthy people, the number of mirror-like tongue, thick tongue coating and the moisture of tongue were increased in cancers. The dominant color of the tongue in the healthy people was reddish while it was purple in cancers. The relative abundance of Neisseria, Haemophilus, Fusobacterium and Porphyromonas in healthy people were higher than that in cancers. They also found 6 kinds of special microorganisms at species level in cancers. The study suggested that tongue coating appearance can be objective assessment and different tongue coating have different microbes. Jie Hu et al[40] found that thick tongue coating presented lower microbial community diversity than thin tongue coating. Many researches have shown that bacteria structure was associated with different tongue coating appearances, but currently there are no related researches on oral bacteria and EGF correlation and their effects on tongue coating formation and change.

In recent years, many scholars have made certain achievements on the tongue coating related research by means of new research methods. Although important progress has been made, there are still many problems. First of all, there are no accordant and impersonal standard for sample collection and tongue image determination. Diseases of different internal organs would be reflected in different regions of tongue coating. The location and time of sampling as well as the cleanliness of the oral cavity will influence experimental results to a certain extent. And now tongue diagnosis still mainly relies on visual observation and experience of doctor judge. It is important to establish an objective standard for tongue coating sample collection and tongue image determination to ensure the comparability of the samples. Secondly, studies were limited in some certain diseases and most data came from blood and saliva sample. For example, existing researches mainly concentrated in blood and saliva of tumor patients or tumor cells, ignoring tongue coating or tongue mucosa tissue which probably provided the more direct evidence. Different diseases have their own inherent characteristics, so it is necessary to collect samples of different diseases for study. Again, the research is not thorough enough. Related research is still in the primary stage, most of which still stays at the levels of the phenomenon, lack of repeated verification and in-depth study. As is well known, tongue coating is the most intuitive and sensitive external reaction of syndrome types and the tongue coating color is the most important factor of tongue image, but the mechanism is not clear. And there is still no research on the relationship between EGF, tongue coating and syndrome types. Finally, the existing research results are not consistent, and if these contradictions are not resolved, the results of these studies will be difficult to be widely recognized and more difficult to be used to guide clinical.

Recently, the rapid development of biological detection technology and information analysis method provides new ideas and new methods for us to systematically study tongue coating principle and the essential law. Progress on objective model of tongue image of human and animal provides a basis for us to carry out more in-depth researches on the theory of tongue diagnosis. All these provide favorable conditions for our follow-up studies. EGF and EGFR are closely related to the occurrence and development of tumors, and at the same time they are closely related to the formation of tongue coating. As a kind of convenient, simple and noninvasive samples, whether tongue coating can be used in early prediction and prognosis of tumors is worthy of increasing number of scholars start to apply various new techniques to the investigation of tongue coating, we can believe that the forming mechanism of tongue coating will be able to obtain new progress and breakthroughs to provid the scientific basis for tongue diagnosis.

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