CANCER CONUNDRUM

MICROBES AND ORAL CANCER

Oral and oropharyngeal squamous cell carcinoma is a heterogeneous group of malignancies that is documented worldwide due in part to the common use of tobacco and alcohol. Globally, approximately 400,000 cases are diagnosed annually with a mortality rate of over 223,000. Furthermore, this group of head and neck cancers ranks among the top ten most common malignancies worldwide (Ferlay *et al*. 2010).[1] However, the incidence of oral cavity carcinoma appears to be increasing in many parts of the world in a manner that it is difficult to explain with traditional risk factors alone.

Meanwhile, interest in the possible relationships between microorganisms and the different stages of cancer development has been rising and numerous mechanisms by which bacteria, viruses and yeast may initiate or promote carcinogenesis are noted. As early as 1868, Bush reported 2 patients with sarcoma that had been infected with Streptococcus and William Coley also detected the presence of Streptococcus pyogenes in a patient suffering from neck cancer. In 1890, the Scottish pathologist William Russell reported circumstantial evidence for the bacterial cause of cancer. One of the mechanisms is direct alteration in the DNA damage response, resulting in the appearance of genetic mutations that accumulate inside the cell and/or the expression of oncogenes that modify cell survival and proliferation.[2]

In particular, the following viruses have been classified by the International Agency for Research on Cancer (IARC) as "carcinogenic to humans": Epstein–Barr virus (EBV), hepatitis B virus (HBV), several types of human papillomavirus (HPV), human T-cell lymphotropic virus type 1 (HTLV-1), hepatitis C virus (HCV), Kaposi's sarcoma associated herpes virus (KSHV), also known as human herpes virus 8 (HHV-8) and human immunodeficiency virus type-1 (HIV-1).[1] Conservative estimates suggest that 12% of the global cancer burden may be attributed to viruses, the percentage being even higher in developing countries. Many bacteria implicated in carcinogenesis are Porphyromonas gingivalis, Exiguobacterium oxidotolerans, Prevotella melaninogenica, Clostridium, *Staphylococcus aureus*, Streptococcus mitis, viridans Streptococci, Veillonella parvula, Micrococcus, Actinomycetes, Bifidobacteria, Lactobacilli and Capnocytophaga species and yeasts like Candida albicans [Figure 1].[3,4]

PARADIGM FOR MICROBIAL CARCINOGENESIS

For establishing the role of microbes in the development of oral cancers, it is essential to identify the organisms that prevail in these tumor specimens and the mechanisms exploited by them for their survival. Various cancers with microbial implication are colorectal cancer caused due to Fusobacterium species, hepatocellular carcinoma due to HPV and EBV infections, lung cancer due to Chlamydia pneumoniae, intestinal

Access this article online	
Quick Response Code:	Website: www.jomfp.in
	DOI: 10.4103/0973-029X.185901

How to cite this article: Bavle RM, Hosthor SS. Cancer conundrum. J Oral Maxillofac Pathol 2016;20:166-9.

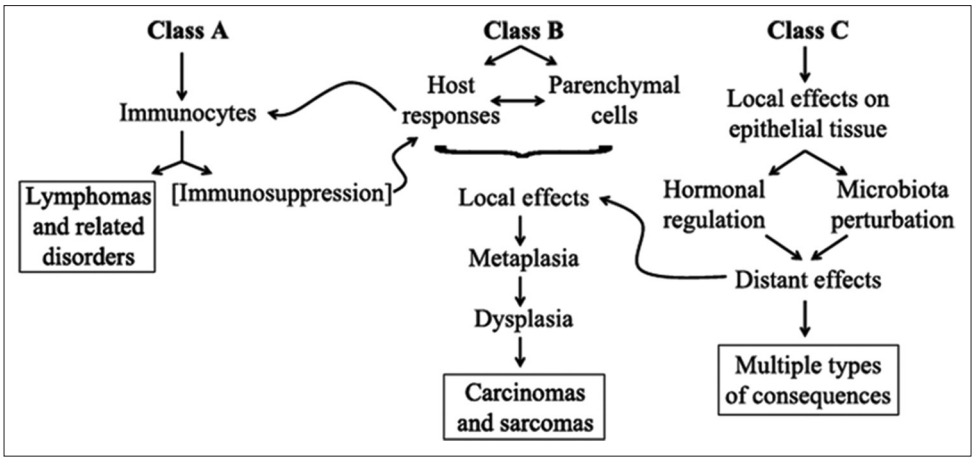

Figure 1: Three classes of microbes that induce cancers. Class A microbes induce cancers including lymphomas by targeting immunocytes leading to immunosuppression. This immunosuppression also contributes to the cancer-inducing effects of Class B microbes, which include local effects on parenchymal cells and induction of host responses. Class C microbes are a postulated class in which a microbe produces local effects on epithelial tissues that change the regulation of a systemic operator (e.g., a hormone) that promotes cancer/degenerative process at a distant site[4]

cancer due to Streptococcus bovis and gastric cancers due to *Helicobacter pylori*. Streptococcus anginosus is linked with esophageal and pharyngeal cancers. Capnocytophaga ochracea, Capnocytophaga gingivalis, Eubacterium saburreum, Leptotrichia buccalis, Candida albicans, P. melaninogenica, and Streptococcus mitis are isolated in oral cancer patients.[3]

Mechanistic basis supporting role of microbes in oral cancer

1. Insertion of additional viral oncogenic genes into the host cell or enhancing already existing oncogenic genes (proto-oncogenes) in the genome by abrogation of tumor suppressor genes (p53, pRb)
2. Inflammation - stimulatory and mutagenic effects of cytokines released by inflammatory cells like reactive oxygen species (ROS), interleukin-8 (IL-8), cyclooxygenase-2 (COX-2) and nitric oxide in response to long term infections like H.pylori
3. Genotoxicity
4. Molecular mimicry
5. Bacterial chemotaxis towards chemo-attractant compounds present in necrotic regions (e.g. aspartate, serine, citrate, ribose or galactose) produced by quiescent cancer cells
6. Microbial toxins - alter host cell cycles or stimulate the production of inflammatory substances like IL-18 and Tumor necrosis factor alpha (TNF α) causing DNA damage.
7. Direct interference with eukaryotic cell cycle and signalling pathways - Mitogen activated kinase (MAPK) pathways and cyclin D1
8. Suppression of apoptosis – modulation of anti-apoptotic proteins (ex: B cell lymphoma 2 (BCL-2) and inactivation of pRb
9. Enzymatic production of metabolic carcinogens such as nitroso compounds and acetaldehyde.[3,5-7]

The oncogenic mechanisms of microbes is presented in Table 1.[5,6,8,9] The tumorigenic and tumoricidal effect of the microbes in oral cancer is depicted using the concept of Yin and Yang in Figure 2. Many studies have demonstrated diverse capabilities and processes that the microbes employ to trick the immune system and relay carcinogenesis. These processes can be turned around to outsmart cancer, thereby highlighting microbial therapeutic applications.

MICROBES IN CANCER THERAPEUTICS

Technical advancements in the field of microbiology like proteomic analysis, nucleic acid sequencing, metagenomic analysis, whole genome sequencing have transformed our ability to use genomic sequence information to explore the origins, evolution, and catalysts associated with historical, emergent and re-emergent disease outbreaks and epidemics like cancers. Several decades after Coley's work a variety of natural and genetically modified microbial species are being explored as potential antitumor agents, either to provide direct tumoricidal effects or to deliver tumoricidal molecules.[6]

BACTERIA AS THERAPEUTIC AGENTS

Bacteria as vector for gene therapy

Genetically engineered bacteria express a specific therapeutic gene and bacterial vectors that can preferentially deliver anticancer agents, cytotoxic peptides, nanosensors, therapeutic proteins or prodrug converting enzymes to solid tumours used for diagnosis and treatment of cancers.

Bacteria as carriers of tumoricidal agents

Bacteria are used for the local delivery of therapeutic cytokines like TNF-alpha and anti-angiogenic proteins like endostatins. Systemic administration of bacterial spores (*ex: Bifidobacterium*

Table 1: Possible oncogenic mechanisms of microbes and its effects

Microbe	Mechanism in oral carcinogenesis	Tumorigenic Effect
Porphyromonas gingivalis	Activates the ERK1/2, p38/HSP27 and PAR2/NF-kB pathways to induce pro-MMP-9 expression Activation of Jak1/Akt/Stat3 signaling, modulation of the expression of Bcl-2 family proteins and inactivation of retinoblastoma protein, pRb	Promotes tumor cell migration, invasion and metastasis (both local and distant) Inhibition of apoptosis
Fusobacterium nucleatum	Targets kinases involved in cell cycle control Production of BA/NaB It also activates β-catenin signaling	Elevates cell proliferation and migration Increased transcriptional activity of oncogenes, Wnt and pro-inflammatory cytokines
Candida albicans	Metabolises ethanol to acetaldehyde using alcohol dehydrogenase	DNA damage, genomic instability, inhibition of apoptosis, mutagenesis, reduced antioxidant activity of glutathione, increased levels of ROS in the cell and secondary hyperproliferation of the epithelium
Herpes simplex virus	Virus associated RNAs lead to defective IFN/PKR pathway, activated Ras protein/Akt pathway, increased EGFR expression	Viral multiplication and spread. proliferation of infected cells prone to mutations. Suppression of apoptosis

ERK: Extracellular signal-regulated kinases, HSP27: Heat shock protein 27, PAR2: Protease-activated receptor 2, NF-kB: Nuclear factor kB, MMP: Matrix metalloproteinase, Jak1/Akt/Stat3: Janus kinase/Protein kinase B/signal transducers and activators of transcription 3, BA: Butyric acid, NaB: Sodium butyrate, IFN/PKR: Interferon/RNA-dependent protein kinase, Ras: Rat sarcoma, EGFR: Epidermal growth factor receptor, ROS: Reactive oxygen species

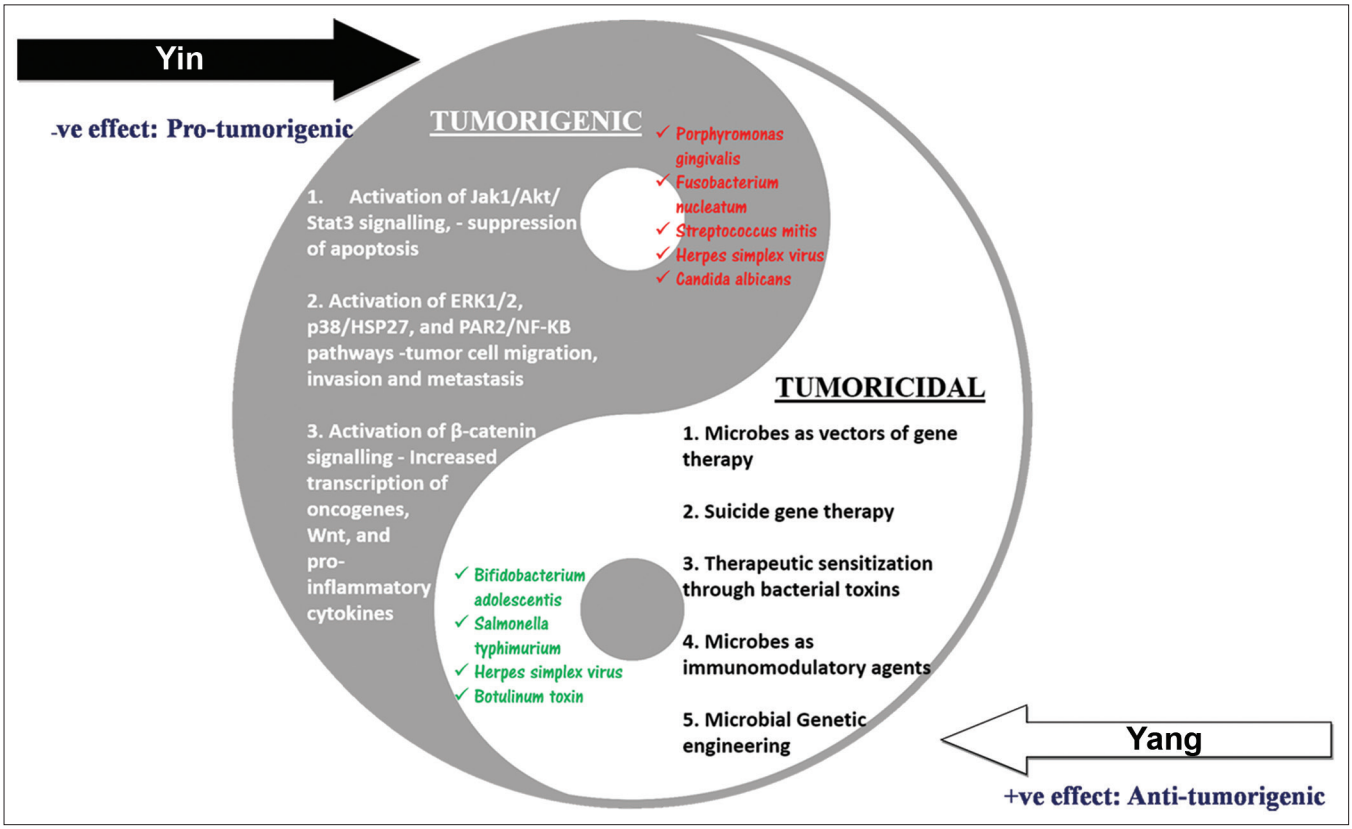

Figure 2: Yin and Yang depicting the tumorigenic and tumoricidal effects of onco-related microbes

adolescentis) can cause a strong inhibition of angiogenesis and reduced tumor growth.

Bacterially directed enzyme prodrug therapy

It uses anaerobic bacteria that have been transformed with an enzyme that can convert a nontoxic prodrug into a toxic drug. *Cytosine deaminase and nitroreductase* are capable of converting prodrug into active anti-tumor drug in the bacterial cell and then it effectively enters the tumor cells and destroys them.

Bacterial toxins for cancer treatment

Toxins have an indirect effect on tumor cells by altering the tumor microenvironment. *Botulinum neurotoxin* opens tumour vessels, allowing more effective destruction of cancer cells by radiotherapy and chemotherapy.

Bacteria as immunotherapeutic agents

Bacterial strains have capacity to modulate immunity to infection and have retarded the growth of tumors. *Attenuated Salmonella*

typhimurium has demonstrated successful invasion of melanoma cells that can present antigenic determinants of bacterial origin and become targets for anti-Salmonella specific T cells.[6]

ONCOLYTIC VIRUSES AS THERAPEUTIC AGENTS

The term 'oncolytic viruses' applies to viruses that are able to replicate specifically in and destroy tumor cells and this preferential trophism is either inherent or genetically-engineered. Inherently tumor-selective viruses can specifically target cancer by exploiting the very same cellular aberrations that occur in these cells, such as surface attachment receptors, activated Ras and Akt, and the defective IFN pathway.[8,9]

Modulations associated with oncolytic viruses are

Gene-manipulated oncolytic viruses

Oncolytic viruses are genetically engineered and designed to have specific tropism, through gene silencing by RNA interference technology, based on the expression of cell surface receptors unique to cancer cells such as in Adenovirus, herpes virus and vaccinia virus. (ex: Talimogene 1 a herparepvec (T-VEC) to treat advanced melanoma).

Selective intratumoral replication

Leads to improved efficacy over non-replicating agents due to the self-perpetuating nature of the treatment with virus multiplication, lysis of the infected tumor and spread to adjacent cells.

Arming oncolytic viruses

Arming with anti-cancer genes has been a major focus in cancer virotherapy and transgenes exploited include tumor suppressor, pro-apoptotic, anti-angiogenic and immunomodulatory genes.

Gene-directed prodrug activation therapy (or suicide gene therapy)

Involves the delivery of a gene that would lead to the expression of an enzyme, followed by the administration of a prodrug that is activated selectively by this enzyme. Example, the herpes simplex virus- thymidine kinase (HSV-TK)-ganciclovir method, whereby HSV-TK is able to monophosphorylate ganciclovir, which is subsequently converted by cellular kinases to the triphosphorylated forms, blocking DNA synthesis and inducing cell death.[8-10]

CONCLUSION AND FUTURE PROSPECTS

There is increasing evidence that pathogenic microbes can contribute to specific stages of cancer development. In particular, chronic infections triggered by microbes can facilitate tumor initiation or progression because, during the course of infection, normal cell functions can be disrupted due to control of factors released by the pathogen. Many of the bacterial and viral infections that promote oncogenesis, by disrupting the oral mucosal surface, allow invasion and perhaps serve as a point of entry to the regional lymph nodes. The multistep nature of microbial oncogenesis provides ample opportunities for interventions to mitigate the process and prevent cancer. However, during the course of evolution the human body has developed ways to overcome infection and this has imposed a significant barrier towards achieving maximum therapeutic efficacy of microbes.[4]

Recent advances in our understanding of tumor biology and virology have helped to successfully target the tumor mechanisms where combination of recombinant DNA technology along with immunotherapy applied to the virus and anaerobic bacteria will serve as the foundation for the multimodality therapeutic strategies for cancer.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Radhika Manoj Bavle, Sreelatha S Hosthor

Department of Oral and Maxillofacial Pathology, Krishnadevaraya College of Dental Sciences, Bengaluru, Karnataka, India.
E-mail: rad.iaomp@gmail.com

REFERENCES

1. Ramirez-Garcia A, Rementeria A, Aguirre-Urizar JM, Moragues MD, Antoran A, Pellon A, *et al.Candida albicans* and cancer: Can this yeast induce cancer development or progression? Crit Rev Microbiol 2016;42:181-93.
2. Khajuria N, Metgud R. Role of bacteria in oral carcinogenesis. Indian J Dent 2015;6:37-43.
3. Chocolatewala N, Chaturvedi P, Desale R. The role of bacteria in oral cancer. Indian J Med Paediatr Oncol 2010;31:126-31.
4. Blaser MJ. Understanding microbe-induced cancers. Cancer Prev Res (Phila) 2008;1:15-20.
5. Whitmore SE, Lamont RJ. Oral bacteria and cancer. PLoS Pathog 2014;10:e1003933.
6. Patyar S, Joshi R, Byrav DS, Prakash A, Medhi B, Das BK. Bacteria in cancer therapy: A novel experimental strategy. J Biomed Sci 2010;17:21.
7. Mohd Bakri M, Mohd Hussaini H, Rachel Holmes A, David Cannon R, Mary Rich A. Revisiting the association between candidal infection and carcinoma, particularly oral squamous cell carcinoma. J Oral Microbiol 2010;2:5780. doi: 10.3402/jom.v2i0.5780.
8. Bartlett DL, Liu Z, Sathaiah M, Ravindranathan R, Guo Z, He Y, *et al.* Oncolytic viruses as therapeutic cancer vaccines. Mol Cancer 2013;12:103.
9. Wong HH, Lemoine NR, Wang Y. Oncolytic viruses for cancer therapy: Overcoming the obstacles. Viruses 2010;2:78-106.
10. Ledford H. Cancer-fighting viruses win approval. Nature 2015;526:622-3.