Meta Gene 5 (2015) 84–89

Contents lists available at ScienceDirect

Meta Gene

Metagenomics: A new horizon in cancer research

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ARTICLE INFO

Article history:
Received 19 March 2015
Revised 21 May 2015
Accepted 26 May 2015
Available online 14 June 2015

Keywords:
Metagenomics
Microbiome
Novel sequencing strategy
Cancer

ABSTRACT

Metagenomics has broadened the scope of targeting microbes responsible for inducing various types of cancers. About 16.1% of cancers are associated with microbial infection. Metagenomics is an equitable way of identifying and studying micro-organisms within their habitat. In cancer research, this approach has revolutionized the way of identifying, analyzing and targeting the microbial diversity present in the tissue specimens of cancer patients. The genomic analyses of these micro-organisms through next generation sequencing techniques invariably facilitate in recognizing the microbial population in biopsies and their evolutionary relationships with each other. In this review an attempt has been made to generate current metagenomic view on cancer microbiota. Different types of micro-organisms have been found to be linked to various types of cancers, thus, contributing significantly in understanding the disease at molecular level.

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Introduction

Cancer is a major health concern in the developed and developing countries. On the World Cancer Day (February 4, 2014), the International Agency for Research on Cancer (IARC) published a worldwide report on cancer in 2012 which estimated about 14.1 million new cancer cases, 8.2 million deaths due to cancer and 32.6 million people living with cancer (International Agency for Research on Cancer, WHO [Internet], 2012). Combined effects of several factors such as genetic, environmental, life style can lead to cancer. One such factor for causing cancer is the cancer induced by microbes which estimates about 16.1% of the total cancer burden globally (De Martel et al., 2012). (See Fig. 1.)

Several studies on microbes and cancers showed distinct associations of various viruses with different types of cancers. Human papilloma virus (HPV) causes cervical cancers (Hausen, 1996) whereas *Helicobacter pylori* induce gastric cancers and Mucosa-associated lymphoid tissue (MALT) lymphoma (Cover and Blaser, 2009). Hepatitis B and C viruses are responsible for Hepatocellular carcinoma (Raza et al., 2007) and Merkel Cell Polyomavirus cause Merkel cell carcinoma (Feng et al., 2008), which is a rare type of skin cancer. Epstein–Barr virus (EBV) has been found to be responsible for Nasopharyngeal carcinoma (NPC), Burkitt's lymphoma,

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http://dx.doi.org/10.1016/j.mgene.2015.05.005

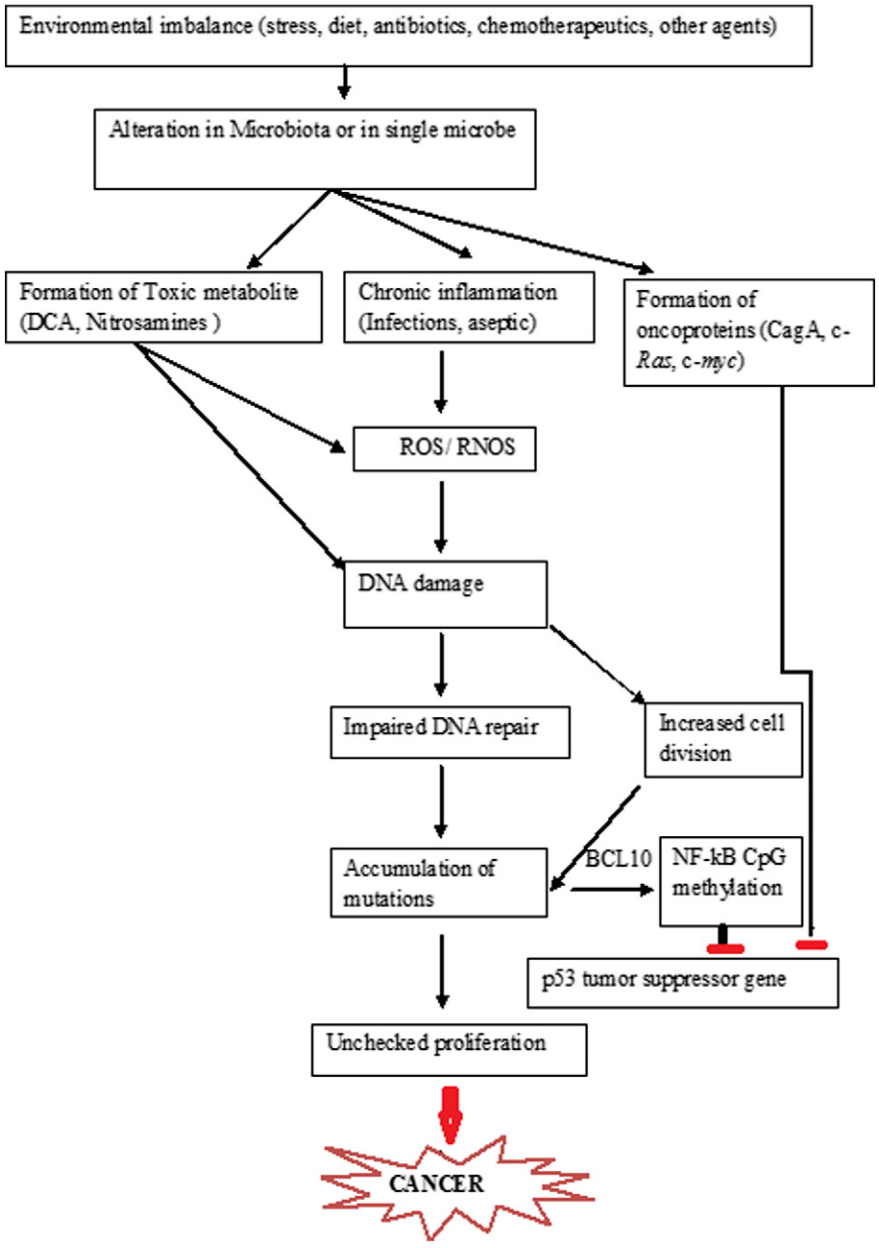

Fig. 1. Mechanism of oncogenesis induced due to change in the microbiota (Chang and Parsonnet, 2010). In this figure, environmental imbalances result in alteration of normal microbiota, subsequent formation of toxic metabolites, chronic inflammation, oncoproteins. The generation of free radicals followed by DNA damage and loss of function of p53 tumor suppressor gene, result in uncontrolled proliferation of cells and formation of cancer. DCA — Deoxycholic acid; BCL10 — B cell lymphoma/leukemia 10 protein; NF-kB — Nuclear Factor kappa B; ROS/RNS — Reactive oxygen species/Reactive nitrogen species.

Hodgkin's lymphoma and to some extent to cause HIV-positive Central nervous system (CNS) lymphomas, hypopharyngeal and laryngeal tumors (Goldenberg et al., 2004).

The studying, analyzing and interpreting of the microbial linkage to cancer has been revolutionized in the emergent era of metagenomics. It is an equitable way of studying culture independent micro-organisms which includes the study of their structures, functions and interactions with their habitat (Handelsman et al, 2007). Various recent researches on cancer due to infection have been explored on the light of genomic analysis of microorganisms residing in the cancerous tissue specimens.

The detailed genomic analysis of microbiota of Colorectal Carcinoma (CRC) reported the presence of various *Fusobacterium* spp. and also species from *Campylobacter* and *Leptotrichia* genera (Castellarin et al., 2012; Kostic et al., 2012; Warren et al., 2013). Further, the metagenomic analysis on the prostate secretions showed the presence of microorganisms belonging to Proteobacteria phylum (Smelov et al., 2014). Metagenomics approach has provided a new way of treating and preventing microbe associated cancers. This review aims to provide extensive studies on the metagenomic approaches concerning microbes induced cancer.

Historical perspectives of microbes inducing cancer

The association of microbes with cancer is not a new fact. The famous experiment of Plymouth Rock hen by Francis Peyton Rous in 1911 evidently proved this fact and subsequently named the virus as Rous sarcoma virus (Rous, 1910, 1911). Rous for his notable tumor inducing RNA virus discovery awarded him Nobel Prize in Medicine in 1966. After this discovery of Rous, the 1930s experienced extensive researches on mammalian tumor virus (Becsei-Kilborn, 2010; Vogt, 1996). In 1964, Anthony Epstein, Bert Achong and Yvonne Barr identified EBV particles in Burkitt's lymphoma cell line derived from African

Table 1
List of microbe associated cancer.

Microbes	Induced cancer	References
Human Papilloma virus	Cervical cancer	Hausen (1996)
Helicobacter pylori	Gastric cancer, MALT lymphoma, Oral cancer	Cover and Blaser (2009); Dayama et al. (2011)
Epstein–Barr virus	Nasopharyngeal carcinoma	Goldenberg et al. (2004)
Merkel Cell Polyomavirus	Merkel cell carcinoma	Feng et al. (2008)
Hepatitis B and C viruses	Hepatocellular carcinoma	Raza et al. (2007)
Human cytomegalo virus	Glioblastoma multiforme	Ranganathan et al. (2012); Soroceanu et al. (2011)
Simian Virus 40	Brain cancer	Pagano et al. (2004)
Streptococcus anginosus	Esophageal cancer, Head and neck cancer	Tateda et al. (2000)
Salmonella typhi	Gall bladder cancer, Cholangiocarcinoma	Lazcano-Ponce et al. (2001); Wistuba and Gazdar (2004); Robbins et al. (1988)
Tropheryma whippelii	Extraintestinal lymphoma Lymphoma and gastric adenocarcinoma	Cadenas et al. (1999); Gillen et al. (1993)
Herpes virus (Kaposi's sarcoma associated Herpes virus)	Kaposi's sarcoma	Ueda (2012)
Mycoplasma penetrans *Mycoplasma tuberculosis*	Kaposi's sarcoma	Barete et al. (2000); Tamburini et al. (2007); Wang et al. (1993)
Chlamydia trachomatis	Epithelial ovarian carcinoma	Quirk and Kupinski (2001)
Chlamydia pneumoniae	Lung carcinoma	Kocazeybek (2003); Koyi et al. (2001); Mager (2006)
Chlamydia psittaci	Ocular lymphoma	Ferreri et al. (2004)

patients by the use of electron microscope (Epstein et al., 1964). The discovery of Harald Zur Hausen on the prevalence of highly risk HPV genotypes in patients' specimens suffering from cervical cancer and strong association of HPV strains with cervical cancer awarded him Nobel Prize in 2008 (Hausen, 1996, 2002). Table 1 contains the list of micro-organisms which are susceptible for causing cancer.

Metagenomics and novel sequencing strategies to target diseases

The complete genome sequencing studies of many pathogenic microorganisms have been enhanced with the outbreak of knowledge about these organisms (Monaghan and Barrett, 2006) and generated plethora of informations about the diseases caused by these organisms. Further, these studies on the micro-organisms contributed in selecting potential antibacterial targets (Sakharkar et al., 2004), pathogenesis (Field et al., 2004; Polissi and Soria, 2005) and antibiotic resistance (Black and Hodgson, 2005). Elucidation on the liaison between the genome sequencing and the microbiome encouraged better understanding of the diseases.

Based on a report by the United States National Research Council committee, "The New Science of Metagenomics: Revealing the Secrets of Our Microbial Planet", metagenomics has revolutionized the research in microbiology and opened up a window for exploring previously unknown world of micro-organisms and their diversity (Handelsman et al., 2007). It has by-passed the need of isolation and lab cultivation of individual species (Chen and Pachter, 2005) and provided the surplus knowledge about the microbial communities and the practical applications from new medical approaches to alternative sources of energy (Jurkowski et al., 2007).

About 99% of the micro-organisms remain uncultured and cannot be demonstrated fully by conventional laboratory culture based techniques (Qin et al., 2010). In metagenomics approach the 16S rRNA gene sequence of bacterial chromosome having highly conserved and variable sequences are used for characterizing microbial communities in diverse conditions (Turnbaugh et al., 2008).

Splendid researches have been carried out on metagenomic analyses of the airborne and soil microbes (Daniel, 2005; Delmont et al., 2011; Yooseph et al., 2013). In recent years, the human microbiota which includes micro-organisms of gut, oral cavity and skin (Grice and Segre, 2012) is much a talked research subject where the role of the microbiome interacting with the human body associated with the development of immune function, disease causation, cancer incidence and defense mechanism against pathogens is vastly studied (Hannigan and Grice, 2013). Moreover, the progress in development of sequencing tools and techniques has helped in investigating human microbiome in various sites of the body (Mardis, 2008).

Recent advancements in the sequence technology have encouraged the microbial genome study and analyses. The next generation sequencing has simplified the process of detection of viral diversity in clinical samples without having prior sequence information (Radford et al., 2012).

Metagenomics and human microbiome

The human intestinal microbiota harbors at least a trillion bacterial cells per gram of feces (Gill et al., 2006). The human gut is the vital center of the body controlling physiology, metabolism, nutrition and immune function. The dysbiosis in the gut microbiome may have direct connection with gastrointestinal conditions such as Crohn's disease, inflammatory bowel disease and obesity (Guinane and Cotter, 2013; Peterson et al., 2008; Tamboli et al., 2004). In a recent study, it has been found that the disruption in the gut microbiota also leads to age related alterations in human (Rampelli et al., 2013).

The human oral cavity like that of human gut, is the dwelling place of hundreds of bacterial species, some of them being the cause of oral diseases like dental caries, periodontal diseases (Marsh, 2010). The oral metagenomic study in Wang et al. (2013) depicted the presence of *Streptococcus* sp., *Haemophilus* sp., *Rothia* sp. and *Capnocytophaga* sp. in the periodontal swab samples and the distinct presence of *Prevotella* sp. forming 14.4–44.7% of the bacterial communities in periodontal disease plaque samples.

Like gut and oral cavity, human skin also harbors varied types of microbes. Disruption in the skin microbiota is responsible for incidence of dermatological diseases (Hannigan and Grice, 2013). The sputum samples of patients suffering from Cystic fibrosis, a genetic disease, have been analyzed for metagenome study, revealed that higher abundance of *S. maltophilia* (41% to 90%) whereas lower incidence of *P. aeruginosa* (<1%) (Lim et al., 2014).

Metagenomic studies on different types of cancers

Gastric carcinoma

The correlations between the microbiota and occurrence of cancers in the liver and gastrointestine have been identified majorly in the recent investigations (De Martel et al., 2012). The gut associated microbes

have been found to be the component for gastrointestinal cancers and the metagenomic profiling of gastrointestinal biopsies revealed the presence of *H. pylori* (Zheng et al., 2011). Low pH in the gastric secretions favors the growth of bacteria and the production of carcinogenic N-nitrosamine compounds. The studies on animal models also confirm the role of gastric microbiota in the development of gastric cancer (Wang et al., 2000). The predominant microbial population in the gastric cancer patients was found to be *Veillonella*, *Haemophilus*, *Streptococcus*, *Lactobacillus*, *Prevotella* and *Neisseria* spp. (Dicksved et al., 2009). The significant difference in the gastric microbiota of patients with *H. pylori* infection and control group without infection revealed the role of *H. pylori* in interfering with the composition of gastric microbiome (Maldonado-Contreras et al., 2010). Eun and his colleagues used 454-high throughput sequencer to confirm the presence of *Helicobacter* spp. in gastric mucosa of patients with gastric cancer and also reported significant difference in the microbiota of patients with chronic gastritis and intestinal metaplasia (Eun et al., 2014).

Colorectal carcinoma

Every year, approximately 1.2 million individuals worldwide are diagnosed with Colorectal Carcinoma (CRC) (Dejea et al., 2013) and the colon is highly exposed to a diverse class of micro-organisms (Warren et al., 2013). Various researches on gut associated microbes, their interactions and incidence of intestinal cancers unravel the accountability of the intestinal microbiome in inducing cancer in human gut. The structural variation in gut microbiota has been found to be responsible for the progression of the CRC (Candela et al., 2014). In 1951, McCoy and Mason put forward the prevalence of *Streptococcus bovis/gallolyticus* in colonic carcinoma, which was traditionally considered to be involved in infectious endocarditis (McCoy and Mason, 1951) until in 1974 when *Streptococcus bovis* was recognized to be associated with the colorectal cancer (Savage, 1977).

The genomic analyses of the tissue samples infected with CRC, carried out by various researchers have been seen to be overpopulated with *Fusobacterium* spp. especially *F. nucleatum*, *F. mortiferum*, *F. necrophorum* along with significant co-occurrence of various anaerobes viz. *Campylobacter* and *Leptotrichia* genera (Castellarin et al., 2012; Kostic et al., 2012; Warren et al., 2013). Although these Gram negative anaerobes are specific to the oral cavity but *Fusobacterium* and *Campylobacter* are genetically diverged in the tumor tissues from their oral counterparts (Warren et al., 2013). *Fusobacterium* spp. also has linkage to inflammatory bowel disease including ulcerative colitis and Crohn's disease (Neut et al., 2002; Strauss et al., 2011) establishing a relatedness of inflammatory bowel disease with CRC.

In another study on CRC, the distinct population of *C. leptum*, *C. coccoides*, Bacteroides/Prevotella, *Lactobacillus/Leuconostoc/Pediococcus* spp., *Bifidobacterium* spp., *E. coli*, and *Faecalibacterium prausnitzii* species have been observed in the tumor samples of patients with colon cancer compared to normal individuals (Sobhani et al., 2011).

Cervical cancer

Cervical cancer, the cancer of the cervix, is the third most common type of cancer of women worldwide with the high mortality rates of 88% in the developing countries (Arbyn et al., 2011). A healthy vaginal microbiota that comprises mainly *Lactobacillus* spp. contributes to women health by lowering pH in vagina through production of lactic acid (Lee et al., 2013). Bacterial Vaginosis (BV) causes the loss of indigenous *Lactobacillus* spp. and the over-growth of anaerobic bacteria instigating imbalance in vaginal microbiota associated with various detrimental health issues like vaginal discharge syndrome, poor pregnancy outcomes, pelvic inflammatory disease, post-operative wound infections and endometritis after elective abortions (Mancuso et al., 2011; Martin, 2012; Ness et al., 2005). The incidence of BV makes an individual to be susceptible more to HPV infection (Gillet et al., 2011).

Persistent HPV infection is the central causative agent for the development and progression of cervical cancer to a higher grade (Koshiol et al., 2008) and only few types of HPV infections possess the risk of cervical cancer (Castellsagué, 2008). HPV can be classified into two types with high-risk type giving rise to cervical carcinoma and low-risk type producing benign warts (Hausen, 2002). There are more than 200 HPV types with 90% of sequence identity in the L1 major capsid region with almost 118 types have their full genome sequenced (Bernard et al., 2010). About 40 types of HPV give rise to anogenital warts with 12 types probably carcinogenic to humans and 12 types possibly carcinogenic to humans as classified by International Agency for Research on Cancer (IARC) (Bouvard et al., 2009). Moreover, HPV-16 and HPV-18 are considered as the most oncogenic types and are almost present in 71% of cases with cervical cancer (De Sanjose et al., 2010).

A comparison study between HPV positive and HPV negative women evaluated by Gao et al. (2013) exhibited an association between vaginal microbiota and the HPV infection. This study unveiled significant presence of *Lactobacillus* spp., including *L. gallinarum*, *L. iners* and *L. gasseri* in all women and *L. gasseri* and *Gardnerella vaginalis* being predominant in women with HPV positive. In another study of detecting diversity in vaginal microbiome among Korean twins by Lee et al. (2013) showed a lowered population of *Lactobacillus* spp. and notable presence of *Fusobacteria* spp., including *Sneathia* spp. in HPV positive cases.

Oral carcinoma

The oral microbiota also plays dramatic effect on the human health. The microbes residing in the oral cavity are often detrimental and give rise to different oral diseases including oral cancers. A well equipped knowledge about the normal microbiota present in the healthy person and the change in the microbial population in the diseased state is the primary requirement (Lazarevic et al., 2009) for targeting the disease. Mager et al. (2005) suggested the fact that salivary micro-organisms can be used as the diagnostic marker for oral cancers and found *Prevotella melaninogenica* and *Capnocytophaga gingivalis* of the Bacteroidetes and *Streptococcus mitis* of the Firmicutes in the Oral Squamous Cell Carcinoma (OSCC). The first metagenomic investigation of the microbiome dwelling in the oral cavity has been carried out to a single sample of healthy individual by next generation sequencing (Xie et al., 2010). Belda-Ferre et al. (2011) used 454-pyrosequencing for studying oral metagenome in various health conditions. The saliva of patients with OSCC mainly comprised large population of Firmicutes and Bacteroidetes and unclassified bacteria and a relatively small percentage of *Mycoplasma* spp. (Tenericutes) were detected by using V4–V5 16S rDNA based 454-parallel DNA sequencing (Pushalkar et al., 2012, 2011).

Brain cancer

Glioblastoma multiforme (GBM) is a most aggressive and malignant, grade 4 type of brain cancer arising from the glial cells or their precursors within the central nervous system with poor prognosis (Holland, 2000; Zhu and Parada, 2002). The polyomavirus simian virus 40 (SV40), a potent oncogenic DNA virus (Butel and Lednicky, 1999), has been found to be associated with the brain cancers (Pagano et al., 2004). In animal models SV40 has been found to induce primary brain neoplasia along with the other types of cancers such as malignant mesotheliomas, bone tumors and systemic lymphomas (Butel and Lednicky, 1999). Ranganathan et al. showed a close association of Human Cytomegalo Virus (HCMV) with the brain cancers. The genomic analysis using quantitative real time PCR of the GBM samples found to comprise all the regions of the genome of HCMV (Ranganathan et al., 2012). A recent study imparted the presence of the CMV sequences and the viral gene expression in most GBM cases (Dziurzynski et al., 2012). A contradictory metagenomic analyses of GBM specimens using High Throughput Sequencing (HTS) and analyses with the multistage computational

pipeline of patient GBM biopsies proclaimed absence of any known viruses despite earlier studies that concluded the strong association of virus with GBM (Cosset et al., 2014). Further, Cosset et al. (2014) revealed the presence of non-specific interferon like pattern analogous to antiviral gene expression.

Skin cancer

The skin that acts as the protective barrier of our body from the external environment comprises large pool of diverse class of micro-organisms. Imbalance in the normal microbial population in the skin may give rise to skin neoplasia. The substantial research investigations confirm the explicit role of micro-organisms even in the non-infectious skin diseases, such as atopic dermatitis, rosacea, psoriasis and acne (Paulino et al., 2006; Till et al., 2000). Gao et al. (2007) used the 16S rRNA sequencing technique for predicting the composition of skin microbiota. Mathieu et al. explored the skin microbiome and conveyed the presence of *Corynebacterium*, *Staphylococcus* and *Propionibacterium* as the dominant skin colonizing taxa (Mathieu et al., 2013), on the other hand, another investigation of the metagenomic analyses of human skin lesions revealed the skin prevalence of 97% of HPV sequences by employing HTS (Bzhalava and Dillner, 2013). A most significant presence of Merkel Cell Polyomavirus (MCPyV) in an aggressive neuro-endocrine skin cancer has been detected (Feng et al., 2008; Shuda et al., 2008) but its co-occurrence in the normal healthy skin surface aroused the questionability of its relatedness towards skin carcinoma (Foulongne et al., 2010; Schowalter et al., 2010; Wieland et al., 2009).

Conclusion

Metagenomics has widened the scope of studying micro-organisms within their habitat. The recent advancements in the field of next generation sequencing techniques have also boosted up this approach. A significant percentage of cancers are induced due to micro-organisms, so metagenomics studies may facilitate the cancer researches by identifying microbes responsible for causing cancer. Finding out potential targets in cancer research will enhance the remedial measures and expand the horizon of cancer research. Thus, future studies are encouraged to explore cancer microbiota for further different types of cancers.

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