CIRUGÍA ESPAÑOLA

www.elsevier.es/cirugia

Review article

Microorganisms and cancer: Scientific evidence and new hypotheses

Encarna Velázquez,[a] *Álvaro Peix,*[b] *Alberto Gómez-Alonso*[c,*]

[a]*Departamento de Microbiología y Genética, Universidad de Salamanca, Salamanca, Spain*
[b]*IRNASA-CSIC, Salamanca, Spain*
[c]*Departamento de Cirugía, Universidad de Salamanca, Salamanca, Spain*

ARTICLE INFORMATION

Article history:
Received January 14, 2010
Accepted August 3, 2010

Keywords:
Cancer
Microorganisms
Humans

A B S T R A C T

Microorganism involvement in cancer has been known for over a century, and different types of parasites, bacteria and viruses have been associated with oncogenic processes. Among the bacteria, the first recognised was Helicobacter pylori which causes gastric cancer and might be related to extra-gastric cancer in humans. *Helicobacter hepaticus* has been associated with liver cancers using animal models. Other bacteria such as, *Chlamydia psitacii*, *Borrelia burgdorferi* and *Streptococcus bovis* have been associated with ocular, skin and colorectal cancers, respectively. Also, a commensal bacterium in the human intestine, *Bacteroides fragilis*, has been linked, very recently, with colorectal cancer using animal models.

Microorganismos y cáncer: evidencias científicas y nuevas hipótesis

Palabras clave:
Cáncer
Microorganismos
Hombre

R E S U M E N

La implicación de los microorganismos en el cáncer humano se conoce desde hace más de un siglo y diferentes tipos de parásitos, bacterias y virus se han relacionado con procesos oncogénicos. Dentro de las bacterias, la primera reconocida como carcinogénica fue Helicobacter pylori, que causa cáncer gástrico y podría estar relacionada con cánceres extragástricos en el hombre. *Helicobacter hepaticus* se ha relacionado con cánceres hepáticos utilizando modelos animales. Otras bacterias, como *Chlamydia psitacii*, *Borrelia burgdorferi* y *Streptococcus bovis*, se han relacionado con cánceres oculares, de piel y colorrectal, respectivamente. Además, una bacteria comensal del intestino humano, *Bacteroides fragilis*, se ha vinculado muy recientemente con el cáncer colorrectal utilizando modelos animales.

*Corresponding author.
E-mail address: agam@usal.es (A. Gómez-Alonso).

0009-739X/$ - see front matter

Introduction

Discovering that microorganisms produce diseases was one of the main milestones for microbiology during the 19th century, and at the end of that century, microbiologists started looking for the origin of many diseases in these organisms, including cancer. Several authors have recently reviewed which microorganisms cause cancer in humans.[1-3] Zur Hausen receives special attention, having been awarded the 2008 Nobel Prize in Medicine for his research on human papillomavirus and its involvement in cervical cancer.[4,5] There is increasing evidence on the involvement of different parasites, viruses and bacterium in human cancer (Table) and the latest research results indicate that more in-depth research on the role of microorganisms is needed.

First were the parasites...

The first human cancer-related microorganisms discovered were different parasites.[4] To be exact, *Opisthorchis felineus* was associated with liver cancer,[6] *Bilharzia* (schistosomiasis) with bladder cancer,[7] and *Spirocerca lupi* with granulomas in dogs which could transform to sarcomas.[8] The International Agency for Research on Cancer (IARC) considered the results from these studies, as well as others, and concluded that there is sufficient evidence that *Schistosoma haematobium* and *Clonorchis viverrini* play a role in human cancer.[9] *Schistosoma haematobium* is currently one of the main causes of gallbladder cancer in Egypt and *Opisthorchis viverrini* and *Clonorchis sinensis* are important factors in bile duct cancer and liver cancer in south east Thailand and southern China.[5]

...Then the viruses...

The next microorganisms found to be involved in different types of tumours were viruses.[4,5] M'Faydan and Hobday published their study on the transmission of warts among animals in 1898.[10] In 1911, Rous showed that a solid tumour, fowl sarcoma, was transmissible with cell-free filtrates.[11] From this point onwards, other viruses were related with tumour development in animals, such as the mammary gland tumour virus in mice,[12] polyomaviruses,[13] a virus which provokes erythroblastosis in adult mice liver,[14] and Simian Virus 40 (from monkey liver) which provoked invasive tumours in very few months when new born hamsters were inoculated.[15,16] Although did the virus not reproduce in these tumours, a specific antigen was produced,[17] as occurred with papillomavirus-induced tumours.[18]

In humans, the first oncogenic virus was described by Burkitt, a surgeon who worked in Africa and detected a lymphoma in children in certain areas of the continent.[19] It was then discovered that the cause was a virus,[20] later named Epstein-Barr virus, responsible for infectious mononucleosis.[21] Immunological techniques were developed to detect viral antigens, meaning that high titres of antibodies could be found in patients with Burkitt's lymphoma[22] and in nasopharyngeal cancers.[23]

During the 1970s, a virus isolated from acute myelogenous leukaemia cells was classified[24] and the presence of mouse

Table – Microorganisms that have been related with different cancers

Microbial species	Cancer location or type	Host
Parasites		
Schistosoma haematobium	Gallbladder	Human
Opisthorchis viverrini	Liver	Human
Clonorchis sinensis	Liver	Human
Bacteria		
Bacteroides fragilis	Colorectal	Mouse
Borrelia burgdorferi	Skin	Human
Chlamydia psittaci	Eye	Human
Helicobacter pylori	Gastrointestinal tract	Human
Helicobacter pylori	Eye	Human
Helicobacter pylori	Breast	Human
Helicobacter hepaticus	Hepatobiliary	Mouse and possibly human
Streptococcus bovis	Intestine	Human
Virus		
Epstein-Barr (EBV)	B-cell, Burkitt and Hodgkin lymphoma, nasopharyngeal cancer	Human
Herpesvirus 8	Kaposi's sarcoma	Human
Papillomavirus	Cervical and other sexual organs	Human
Hepatitis B and C virus (HBV and HCV)	Liver	Human
HTLV-1 virus	Leukaemia	Human
SV40 virus	Liver	Monkey, mouse, and hamster
Mouse mammary tumour virus (MMTV)	Breast	Mouse

mammary tumour virus was found in breast milk and breast cancer.[25] Later, hepatitis B was found to be involved in liver cancer,[26] retroviruses were identified in a rare form of human leukaemia,[27] papillomavirus was found to be involved in female cervical cancer,[4,5] hepatitis C in liver cancer,[28] and herpes virus 8 as the most likely Kaposi's sarcoma agent.[29]

...And finally the bacteria

Although bacteriology evolved much sooner than virology, the latest microorganisms found to be involved in human cancer were bacteria. It was not until 1905 that the first results on the isolation of a tumour-derived bacteria were published, which the surgeon Doyen[30] called *Micrococcus neoformans*. He even prepared a bacterial vaccine, which was thought to have cured cancer. It was applied by Wright, who described that it treated a case of inoperable cancer.[31] The Wright group observed that this bacterium's characteristics were compatible with the *Staphylococcus* genus.[32] Obviously, the cancer diagnostic and bacteria identification techniques used at that time were not reliable enough to ensure that *Staphylococcus* genus bacteria was involved in cancer, even though it may cause infections in cancer patients,[33,34] and have been isolated, with other bacteria, in solid tumours, such as breast tumours.[35]

The interest that awoke with regard the implication of several viruses in different cancers forced the study on bacteria involvement in these diseases to be postponed. It was not until the end of the 20th century that the first carcinogenic bacterium was clearly related to human tumour development, being *Helicobacter pylori*. Its implication in gastric cancer was discovered in 1991,[36-39] and in 1994, *H. pylori* was recognised as a carcinogenic agent.[9] Some years later, experts found that the gastric cancer-producing capability was related to the presence of certain regions in the bacterium genome, called pathogenicity islands. They are called so because at their ends they have direct-repeat DNA sequences that separate them from the rest of the genome. These regions are absent in non-pathogenic strains, which can acquire them by genetic transfer.[40,41] The *Helicobacter pylori* islands belong to the type IV secretion system,[42,43] present in other pathogenic bacteria such as *Agrobacterium tumefaciens* and responsible for tumour formation in higher plants.[44]

Since *H. pylori* was discovered to be involved in gastric cancer, several bacteria have been identified in different types of tumours. However, experts have yet been able to show whether they are a direct cause of carcinogenesis, as in the case of *Chlamydia psittaci* and various types of eye cancers,[45] *Borrelia burgdorferi* and skin lymphomas,[46] different species of *Streptococcus* and colon cancer and other gastrointestinal cancers,[47,48] and *Bacteroides fragilis* and colorectal cancer.[49]

Koch's postulates in cancer

Research on the bacteria that may be involved in cancer has continued during the 21st century. It mainly focuses on *Helicobacter pylori*, on which numerous reviews have recently been published.[50-52] It is difficult to show that a microorganism is capable of inducing cancer, given that an infectious agent may trigger the initial mechanisms of oncogenesis, but be absent in the final tumour.[1,5] Since Robert Koch formulated his infamous postulates, which must be met to ensure that a microorganism is the cause of an infectious process, only *Helicobacter pylori* has been proven in human beings, and only in cases of gastritis. And even so, it was not until a century after this bacterium was discovered in human stomach ulcers[53] that Marshall was able to prove Koch's postulates.[54] However, this bacterium was recognised as being a category 1 carcinogen only 9 years later.[9] At present, Koch's postulates are only met in animal models, but on many occasions the causal microorganism can not be isolated, meaning that experts have to recourse to examining the microbial genes present in cancerous tissues. Koch's postulates may therefore have to be redefined in the event that microorganisms are not found at tumour detection.

Bacteria involved in gastrointestinal cancer

After two decades of research, the role of *H. pylori* in certain types of gastric cancer is widely accepted, including bacterium eradication as part of its treatment.[55-57] Several studies have been performed to try and establish this bacterium's specific mechanisms of interaction with humans,[58,59] virulence factors,[60,61] and to which secretion system its pathogenicity islands belong.[62,63] The risk of *H. pylori*-induced gastric cancer is greater for patients infected with strains that carry the cagA gene in a pathogenicity island.[64] Furthermore, given that this island is widely distributed in people infected with *H. pylori,* these findings are not very reliable and it seems that there are differences between types of cancer. The relationship is more evident between strains with the island and gastric tumours which are morphologically similar to intestinal tissue as associated with p53 mutations found in intestinal cancer.[65] However, both strains with and without the island can be involved in diffuse gastric cancers.[66] Furthermore, certain alleles of the vacA gene, mainly involved in gastritis, are also related with gastric cancer.[67,68] Variations in cag gene sequences from the *H. pylori* island have been found in some populations, meaning that it would be difficult to use in diagnosing the virulent strain of this bacterium.[69,70] However, it is clear that the presence of this whole island in *H. pylori* is related to more severe gastric symptoms.[71] Gastrin has also recently been confirmed as being an essential cofactor in *H. pylori*-induced gastric cancers in animal models.[72]

Given the severity and the increase in gastric cancer during the past decade in some regions of the world,[73] including *H. pylori*-induced gastric cancer, eradication of this bacterium has been proposed for all patients with *H. pylori*. This is the case even if cancer has not developed as it a long process that may only express itself as atrophic gastritis[74] during the first stages. Furthermore, almost all patients with mucosa-associated lymphoid tissue (MALT) gastric lymphomas were cured with this treatment.[57] However, in

other types of gastric cancer, bacterium eradication only reduces its prevalence by a third.[75] Bacterium eradication is not achieved in all cases, and patients infected with strains with vacA and cagA pathogenicity islands have greater risk of eradication failure.[61] As such, numerous studies have been conducted on the mechanisms involved in the host immune response towards the infection,[76] making it easier to promote a vaccine which prevents *Helicobacter* pylori-induced cancers.[77]

Considering the relationship between *H. pylori* and stomach cancer, it is possible that this bacterium is involved in gastrointestinal tract organs, given that it has been found in human bile and the gallbladder.[78] However, more studies need to be conducted and more protocols standardised for detecting DNA or even anti-*Helicobacter* antibodies in bacteria, before being able to associate this bacterium with biliary tract cancers.[79,80] Several studies have found *H. pylori* DNA in human liver cancers, but the exact *Helicobacter* species was not established in some cases.[81-83] Another species of the *Helicobacter* genus, *H. hepaticus*, has been involved in hepatobiliary cancer in animal models.[78,79,84,85] Very recently, using molecular biology and immunology techniques, *H. hepaticus* has been described as being present in the gallbladder in patients with different digestive problems, including gastric cancer.[86] The results were contradictory for pancreas cancer, although a positive relationship has been found between the presence of *H. pylori* and this type of tumour in never smokers and subjects with a low alcohol consumption.[87] Results show that there is no relationship with this bacterium for the oesophagus and larynx.[88,89]

The increase in the risk of suffering from colorectal cancer has been associated with infection of several microorganisms,[90] among which *H. pylori*[91] and *Streptococcus bovis*[92] must be highlighted in humans, and *H. hepaticus* in mice.[93] However, more studies must be conducted so as to clarify whether the latter species may cause this type of cancer in humans.[94] *Streptococcus bovis* has been associated with colorectal cancer since the 1970s,[47,95] however, some of this species strains have been reclassified as *S. infantarius* and *S. gallolyticus*.[96] In accordance with the epidemiological studies, a very high association has been found between *S. gallolyticus* and colon cancer, while *S. infantarius* has a greater correlation with cancer in other gastrointestinal tract organs, such as the pancreas and bile ducts.[96] Other more recent studies have found that *S. gallolyticus* plays a vital role in the progression of normal colorectal mucosa to adenoma and colorectal cancer.[97] Given that *Streptococcus*-induced endocarditis are associated with colorectal cancer,[47,48,95] colonoscopy has been reported to be mandatory for patients with endocarditis caused by these microorganisms.[98]

A study has recently been published on *Bacteroides fragilis*-induced tumours. It is a commensal bacterium from the human intestine, whose role in colorectal cancer may be similar to that of *H. pylori* in gastric cancer. Evidence has been found that the enterotoxigenic strains of this bacterium produce colitis and induce tumour in murine colon models, by activating T helper cells which could also be involved in producing cancer in humans.[49]

Bacteria involved in extra-gastric cancers

Staphylococcus aureus could be considered as the first bacterium to be described as a cancer-producing agent, and some authors have attempted to associate it with breast cancer,[32] however, its involvement in human cancer has never been shown. Furthermore, in a recent study using cell cultures, an extracellular protein involved in *S. aureus* adherence has proven to maybe prevent bone metastasis in breast cancer.[99] However, *S. aureus*-induced infection have recently been described in breast cancer cases in which the relationship between both illnesses was not clear.[100] Furthermore, type 16 papillomavirus (HPV-16) has also recently been found in the genome of different bacteria isolated from cervical cancer, including *Staphylococcus aureus*.[101] The authors suggest that the presence of these viruses in bacterial genome could explain the progression of a HPV-16-induced infection to cervical cancer, using the bacterium as a vector.[101]

Recent studies have also associated *H. pylori* with extra-gastric cancers, such as lung and breast cancer,[102-104] mainly by gastrin induction, which, apart from being a hormone, is a growth factor involved in carcinogenesis and metastasis of these two types of tumours.[102,103] Stress together with mast cells located in the blood-brain barrier may trigger a series of reactions which promote metastatic brain tumours originating in lung and breast cancer.[105] *H. pylori* is involved in this process and it has been suggested that its eradication could prevent this type of brain metastasis.[106]

The presence of *Borrelia burgdorferi* DNA in certain lymphomas has lead to suggesting an association between this bacterium and non-Hodgkin skin lymphomas.[46] This bacterium may survive on patients' skin for decades, and occasionally, it can develop into B-cell lymphomas and other carcinogenic neoplasms, meaning that *B. burgdorferi* has been suggested to be related to this type of tumour.[107]

In recent years, different bacteria have also been related with some types of eye cancer,[108] among which we can highlight *H. pylori* and *Chlamydia*. Contradictory studies have been found for MALT-type eye cancers with regards *H. pylori* involvement: some studies suggesting that this bacterium is involved,[109,110] while others only show negative results.[111] It seems that *Chlamydia* is most likely to be involved in eye cancers and therefore its eradication, and that of *H. pylori*, has been recommended before indicating more aggressive therapies.[46,109] *Chlamydia psittaci* is currently the only species identified in MALT-type eye cancers.[109,111,112] Some authors have found geographical differences,[111] with a positive relationship between this bacterium and eye cancers in Italy[46] and Austria,[112] and a negative one in the United States.[113] It is believed that these conflicts could be due to the bacteria detection methodology used.[114] Some authors have found that another species, *Chlamydia trachomatis*, could be a risk factor in the presence of papillomavirus in some types of carcinomas.[115] It seems that this bacterium could induce an inflammatory response in ovarian cancer, which would lead to different types of cancer, although authors recommend more in-depth studies.[116]

Metagenomics: a new method for detecting tumour-producing bacteria

It is currently accepted that bacteria may be involved in different types of cancers. However, it is not easy to detect them[1] due to multiple causes, including that cancer is not the result of an acute infection, and therefore the causal agent may not be extracted from the tumour.[5] However, viral or bacterial DNA can persist during a rather long period of time, either in the tumour itself, or in the peritumoral area. Therefore, molecular techniques based on bacterial DNA amplification in tumour tissues are the most commonly applied for detecting and identifying bacteria in tumours. Detection techniques have also been proposed for "finding" exogenous DNA sequences after sequencing fragments of the tumour's DNA.[117-119] We must assume that Koch's postulates are not going to be met very often, because even if we were to use pure cultures, the microorganisms' carcinogenic effect must be confirmed using animal models, such as occurred recently with *Bacteroides fragilis,* whose carcinogenic role in humans has been suggested using findings from murine models.[49]

Molecular biology techniques which allow non-isolated microorganisms to be identified are known as metagenomics, and are based on amplifying microbial genes directly from a sample. Subsequent sequencing identifies which microorganisms are in the sample.[120,121] Some metagenomic techniques have an advantage as they can examine the microorganisms in complex ecosystems, such as the oral cavity[122] or intestine[123]. To analyse this type of sample, various techniques can be applied such as denaturing gradient gel electrophoresis (DGGE)[124] based on different electrophoretic mobility due to changes in the denaturing pattern, or single-strand conformation polymorphism (SSCP),[125] based on the secondary conformation of the single-strand DNA chains for the ribosomal 16S gene, whose sequence is the base for classifying and identifying bacteria. Furthermore, to analyse complex populations, the intergenic transcribed spacer (ITS) regions between the ribosomal 16S and 23S genes in bacteria, and between 18S and 28S in fungi are especially useful, which may be separated electrophoretically by the ribosomal intergenic spacer analysis (RISA) technique. The size of ITS in bacteria is very varied, and the RISA technique can separate the ITS for most bacterial groups.[126] Subsequent sequencing of the separated fragments allows bacteria identification, given that they are sequenced in all pathogenic bacteria, and in the main human commensal bacteria.[126] Intestinal bacteria populations have been analysed using this technique in colorectal[127] and laryx[126] cancer patients.

An advantage associated with metagenomic techniques is that they can identify culturable and non-culturable microorganisms present in human microbiome of healthy individuals and those affected by tumoral processes.[128,129] During these processes, changes occur in the microbiome originating from the tumour process,[129] antibiotic treatment[130,131] or radiological treatment and chemotherapy.[130,132] As a result, metagenomics is a promising tool for investigating which microorganisms are present in tumours, given that millions of sequences can be analysed using next-generation sequencing techniques at record speed and at much more competitive prices. There is no doubt that this type of technique, which can detect microbial genes in any sample, will contribute substantially to finding microorganisms involved in tumour formation.

Conflict of interest

The authors affirm that they have no conflict of interest.

REFERENCES

1. Dalton-Griffin L, Kellam P. Infectious causes of cancer and their detection. J Biol. 2009;8:67.
2. De Martel C, Franceschi S. Infections and cancer: established associations and new hypotheses. Crit Rev Oncol Hematol. 2009;70:183-94.
3. Ziegler JL, Buonaguro FM. Infectious agents and human malignancies. Frontiers Biosci. 2009;14:3455-64.
4. Zur Hausen H. Infections causing human cancer. 2006. Germany: Whiley-VCH Verlag GmbH & Co; 2006.
5. Zur Hausen H. The search for infectious causes of human cancers: where and why (Nobel lecture). Angew Chem Int Ed Engl. 2009;48:5798-808.
6. Askanazy M. Über infektion des Menschen mit Distomum felineum (sibiricum) in Ostpreussen und ihren Zusammenhang mit Leberkrebs. Cent Bakt Orig. 1900;28:491-502.
7. Goebel C. Über die bie Bilharziakrankheit vorkommenden Blasentumoren mit besonderer Berücksichtigung des Carcinoms. Zeitschr Krebsforsch. 1905;3:369-513.
8. Bailey WS. Parasites and cancer: sarcoma in dogs associated with Spirocerca lupi. Ann N Y Acad Sci. 1963;108:890-923.
9. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June Schistosomes, liver flukes and Helicobacter pylori. IARC Monogr Eval Carcinog Risks Hum. 1994;61:1–241.
10. M'Faydan J, Hobday F. Note on the experimental transmission of warts in the dog. J Comp Pathol Ther. 1898;11:341-4.
11. Rous P. A sarcoma of the fowl transmissible by an agent separable from the tumour cells. J Exp Med. 1911;13: 397-411.
12. Bittner JJ. Some possible effects of nursing on the mammary gland tumor incidence in mice. Science. 1936;84:162.
13. Stewart SE. Polyoma virus carcinogenesis. Acta Unio Int Contra Cancrum. 1963;19:255-62.
14. Friend C. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. J Exp Med. 1957;105:307-18.
15. Eddy BE, Grubbs GE, Young RD. Tumor immunity in hamsters infected with adenovirus type 12 or simian virus 40. Proc Soc Exp Biol Med. 1964;117:575-9.

16. Girardi AJ, Sweet BH, Slotnick VB, Hilleman MR. Development of tumors in hamsters inoculated in the neonatal period with vacuolating virus, SV-40. Proc Soc Exp Biol Med. 1962;109:649-60.
17. Black PH, Rowe WP, Turner HC, Huebner RJ. A specific complement-fixing antigen present in SV40 tumor and transformed cells. Proc Natl Acad Sci U S A. 1963;50:1148-56.
18. Habel K. Specific complement-fixing antigens in polyoma tumors and transformed cells. Virology. 1965;25:55-61.
19. Burkitt D. A children's cancer dependent on climatic factors. Nature. 1962;194:232-4.
20. Epstein MA, Achong BG, Barr YM. Hair-Sprays. Lancet. 1964;1:709-10.
21. Henle G, Henle W, Diehl V. Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. Proc Natl Acad Sci U S A. 1968;59:94-101.
22. Henle W, Hummeler K, Henle G. Antibody coating and agglutination of virus particles separated from the EB3 line of Burkitt lymphoma cells. J Bacteriol. 1966;92:269-71.
23. Old LJ, Boyse EA, Oettgen HF, De Harven E, Geering G, Williamson B, et al. Precipitating antibody in human serum to an antigen present in cultured Burkkit's lymphoma cells. Proc Natl Acad Sci USA. 1966;56:1699-705.
24. Gallagher RE, Gallo RC. Type C RNA tumor virus isolated from cultured human acute myelogenous leukemia cells. Science. 1975;187:350-3.
25. Das MR, Vaidya AB, Sirsat SM, Moore DH. Polymerase and RNA studies on milk virions from women of the Parsi community. J Natl Cancer Inst. 1972;48:1191-6.
26. Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. Lancet. 1981;2:1129-33.
27. Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita KI, et al. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc Natl Acad Sci U S A. 1981;78:6476-80.
28. Simonetti RG, Cottone M, Craxi' A, Pagliaro L, Rapicetta M, Chionne P, et al. Prevalence of antibodies to hepatitis C virus in hepatocellular carcinoma. Lancet. 1989;2:1338.
29. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science. 1994;266:1865-9.
30. Doyen TA. On the aetiology and treatment of cancer. Edinburgh Med J. 1905;17:373-8.
31. Spicer S, Wright AE. Case of inoperable cancer of the fauces, the pharinx, the tongue and the cervical glands that has shown marked amelioration after treatment for ten weeks with a bacterial vaccine to neoformans. J Laryngol. 1906;21:265-9.
32. Wainwright M. Highly pleomorphic staphylococci as a cause of cancer. Med Hypotheses. 2000;54:91-4.
33. Felippe WA, Werneck GL, Santoro-Lopes G. Surgical site infection among women discharged with a drain in situ after breast cancer surgery. World J Surg. 2007;31:2293-9.
34. Fukushima T, Kasai Y, Kato K, Fujisawa K, Uchida A. Intradural squamous cell carcinoma in the sacrum. World J Surg Oncol. 2009;7:16.
35. Brook I. Bacteria from solid tumours. J Med Microbiol. 1990;32:207-10.
36. Forman D. Helicobacter pylori infection: a novel risk factor in the etiology of gastric cancer. J Natl Cancer Inst. 1991;83:1702-3.
37. Nomura A, Stemmermann GN, Chyou PH, Kato I, Pérez Pérez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med. 1991;325:1132-6.
38. Parsonnet J, Vandersteen D, Goates J, Sibley RK, Pritikin J, Chang Y. Helicobacter pylori infection in intestinal- and diffuse-type gastric adenocarcinomas. J Natl Cancer Inst. 1991;83:640-3.
39. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet. 1991;338:1175-6.
40. Gal-Mor O, Finlay BB. Pathogenicity islands: a molecular toolbox for bacterial virulence. Cell Microbiol. 2006;8: 1707-19.
41. Juhas M, van der Meer JR, Gaillard M, Harding RM, Hood DW, Crook DW. Genomic islands: tools of bacterial horizontal gene transfer and evolution. FEMS Microbiol Rev. 2009;33:376-93.
42. Oliveira MJ, Costa AC, Costa AM, Henriques L, Suriano G, Atherton JC, et al. Helicobacter pylori induces gastric epithelial cell invasion in a c-Met and type IV secretion system-dependent manner. J Biol Chem. 2006;281:34888-96.
43. Pinto-Santini DM, Salama NR. Cag3 is a novel essential component of the Helicobacter pylori Cag Type IV secretion system outer membrane subcomplex. J Bacteriol. 2009;191:7343-52.
44. Juhas M, Crook DW, Hood DW. Type IV secretion systems: tools of bacterial horizontal gene transfer and virulence. Cell Microbiol. 2008;10:2377-86.
45. Ferreri AJ, Ponzoni M, Guidoboni M, De Conciliis C, Resti AG, Mazzi B, et al. Regression of ocular adnexal lymphoma after Chlamydia psittaci-eradicating antibiotic therapy. J Clin Oncol. 2005;23:5067-73.
46. Schöllkopf C, Melbye M, Munksgaard L, Smedby KE, Rostgaard K, Glimelius B, et al. Borrelia infection and risk of non-Hodgkin lymphoma. Blood. 2008;111:5524-9.
47. Klein RS, Recco RA, Catalano MT, Edberg SC, Casey JI, Steigbigel NH. Association of Streptococcus bovis with carcinoma of the colon. N Engl J Med. 1977;297:800-2.
48. Kim SY, Joo SI, Yi J, Kim EC. A Case of Streptococcus gallolyticus subsp. gallolyticus infective endocarditis with colon cancer: identification by 16S ribosomal DNA sequencing. Korean J Lab Med. 2010;30:160-5.
49. Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med. 2009;15:1016-22.
50. Costa AC, Figueiredo C, Touati E. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2009;14:15-20.
51. Hatakeyama M. Helicobacter pylori and gastric carcinogenesis. J Gastroenterol. 2009;44:239-48.
52. Suzuki H, Iwasaki E, Hibi T. Helicobacter pylori and gastric cancer. Gastric Cancer. 2009;12:79-87.
53. Bottcher A. Zur Genese des perforierenden Magengeschwurs. Dopater Medicinische Zeitschrift. 1874;5:148.
54. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric Campylobacter. Med J Aust. 1985;142:436-9.
55. Fuccio L, Zagari RM, Eusebi LH, Laterza L, Cennamo V, Ceroni L, et al. Meta-analysis: can Helicobacter pylori eradication treatment reduce the risk for gastric cancer? Ann Intern Med. 2009;151:121-8.
56. Wu CY, Kuo KN, Wu MS, Chen YJ, Wang CB, Lin JT. Early Helicobacter pylori eradication decreases risk of gastric cancer in patients with peptic ulcer disease. Gastroenterology. 2009;137:1641.e1–1648.e2

57. Zullo A, Hassan C, Andriani A, Cristofari F, De Francesco V, Ierardi E, et al. Eradication therapy for Helicobacter pylori in patients with gastric MALT lymphoma: a pooled data analysis. Am J Gastroenterol. 2009;104:1932-7.
58. Beswick EJ, Suárez G, Reyes VE. H. pylori and host interactions that influence pathogenesis. World J Gastroenterol. 2006;12:5599-605.
59. Amieva MR, El-Omar EM. Host-bacterial interactions in Helicobacter pylori infection. Gastroenterology. 2008;134:306-23.
60. Romano M, Ricci V, Zarrilli R. Mechanisms of disease: Helicobacter pylori-related gastric carcinogenesis–implications for chemoprevention. Nat Clin Pract Gastroenterol Hepatol. 2006;3:622-32.
61. Sugimoto M, Yamaoka Y. Virulence factor genotypes of Helicobacter pylori affect cure rates of eradication therapy. Arch Immunol Ther Exp (Warsz). 2009;57:45-56.
62. Bourzac KM, Guillemin K. Helicobacter pylori-host cell interactions mediated by type IV secretion. Cell Microbiol. 2005;7:911-9.
63. Snaith A, El-Omar EM. Helicobacter pylori: host genetics and disease outcomes. Expert Rev Gastroenterol Hepatol. 2008;2:577-85.
64. Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. Gastroenterology. 2003;125:1636-44.
65. Shibata A, Parsonnet J, Longacre TA, García MI, Puligandla B, Davis RE, et al. CagA status of Helicobacter pylori infection and p53 gene mutations in gastric adenocarcinoma. Carcinogenesis. 2002;23:419-24.
66. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut. 1997;40:297-301.
67. Garza-González E, Bosques-Padilla FJ, Pérez-Pérez GI, Flores-Gutiérrez JP, Tijerina-Menchaca R. Association of gastric cancer. HLA-DQA1, and infection with Helicobacter pylori CagA+ and VacA+ in a Mexican population. J Gastroenterol. 2004;39:1138-42.
68. Basso D, Zambon CF, Letley DP, Stranges A, Marchet A, Rhead JL, et al. Clinical relevance of Helicobacter pylori cagA and vacA gene polymorphisms. Gastroenterology. 2008;135:91-9.
69. Baghaei K, Shokrzadeh L, Jafari F, Dabiri H, Yamaoka Y, Bolfion M, et al. Determination of Helicobacter pylori virulence by analysis of the cag pathogenicity island isolated from Iranian patients. Dig Liver Dis. 2009;41:634-8.
70. Ali M, Khan AA, Tiwari SK, Ahmed N, Rao LV, Habibullah CM. Association between cag-pathogenicity island in Helicobacter pylori isolates from peptic ulcer, gastric carcinoma, and non-ulcer dyspepsia subjects with histological changes. World J Gastroenterol. 2005;11: 6815-22.
71. Talarico S, Gold BD, Fero J, Thompson DT, Guarner J, Czinn S, et al. Pediatric Helicobacter pylori isolates display distinct gene coding capacities and virulence gene marker profiles. J Clin Microbiol. 2009;47:1680-8.
72. Takaishi S, Tu S, Dubeykovskaya ZA, Whary MT, Muthupalani S, Rickman BH, et al. Gastrin is an essential cofactor for Helicobacter-associated gastric corpus carcinogenesis in C57BL/6 mice. Am J Pathol. 2009;175: 365-75.
73. Quirós RM, Bui CL. Multidisciplinary approach to esophageal and gastric cancer. Surg Clin North Am. 2009;89:79-96.
74. Graham DY, Asaka M. Eradication of gastric cancer and more efficient gastric cancer surveillance in Japan: two peas in a pod. J Gastroenterol. 2010;45:1-8.
75. Ito M, Takata S, Tatsugami M, Wada Y, Imagawa S, Matsumoto Y, et al. Clinical prevention of gastric cancer by Helicobacter pylori eradication therapy: a systematic review. J Gastroenterol. 2009;44:365-71.
76. Suárez G, Reyes VE, Beswick EJ. Immune response to H. pylori. World J Gastroenterol. 2006;12:5593-8.
77. Del Giudice G, Malfertheiner P, Rappuoli R. Development of vaccines against Helicobacter pylori. Expert Rev Vaccines. 2009;8:1037-49.
78. Pandey M, Shukla M. Helicobacter species are associated with possible increase in risk of hepatobiliary tract cancers. Surg Oncol. 2009;18:51-6.
79. De Martel C, Plummer M, Parsonnet J, Van Doorn LJ, Franceschi S. Helicobacter species in cancers of the gallbladder and extrahepatic biliary tract. Br J Cancer. 2009;100:194-9.
80. Xuan SY, Xin YN, Chen AJ, Dong QJ, Qiang X, Li N, et al. Association between the presence of H. pylori in the liver and hepatocellular carcinoma: a meta-analysis. World J Gastroenterol. 2008;14:307-12.
81. Abu Al-Soud W, Stenram U, Ljungh A, Tranberg KG, Nilsson HO, Wadström T. DNA of Helicobacter spp. and common gut bacteria in primary liver carcinoma. Dig Liver Dis. 2008;40:126-31.
82. Avenaud P, Marais A, Monteiro L, Le Bail B, Bioulac Sage P, Balabaud C, et al. Detection of Helicobacter species in the liver of patients with and without primary liver carcinoma. Cancer. 2000;89:1431-9.
83. Pellicano R, Mazzaferro V, Grigioni WF, Cutufia MA, Fagoonee S, Silengo L, et al. Helicobacter species sequences in liver samples from patients with and without hepatocellular carcinoma. World J Gastroenterol. 2004;10:598-601.
84. Diwan BA, Sipowicz M, Logsdon D, Gorelick P, Anver MR, Kasprzak KS, et al. Marked liver tumorigenesis by Helicobacter hepaticus requires perinatal exposure. Environ Health Perspect. 2008;116:1352-6.
85. Canella KA, Diwan BA, Gorelick PL, Donovan PJ, Sipowicz MA, Kasprzak KS, et al. Liver tumorigenesis by Helicobacter hepaticus: considerations of mechanism. In Vivo. 1996;10:285-92.
86. Hamada T, Yokota K, Ayada K, Hirai K, Kamada T, Haruma K, et al. Detection of Helicobacter hepaticus in human bile samples of patients with biliary disease. Helicobacter. 2009;14:545-51.
87. Lindkvist B, Johansen D, Borgström A, Manjer J. A prospective study of Helicobacter pylori in relation to the risk for pancreatic cancer. BMC Cancer. 2008; 8:321.
88. Masoud N, Manouchehr K, Najmeh D, Monireh H. Lack of association between Helicobacter pylori and laryngeal carcinoma. Asian Pac J Cancer Prev. 2008;9:81-2.
89. Rezaii J, Tavakoli H, Esfandiari K, Ashegh H, Hasibi M, Ghanei G, et al. Association between Helicobacter pylori infection and laryngo-hypopharyngeal carcinoma: a case-control study and review of the literature. Head Neck. 2008;30:1624-7.
90. Burnett-Hartman AN, Newcomb PA, Potter JD. Infectious agents and colorectal cancer: a review of Helicobacter pylori, Streptococcus bovis, JC virus, and human papillomavirus. Cancer Epidemiol Biomarkers Prev. 2008;17:2970-9.

91. Zhao YS, Wang F, Chang D, Han B, You DY. Meta-analysis of different test indicators: Helicobacter pylori infection and the risk of colorectal cancer. Int J Colorectal Dis. 2008;23:875-82.
92. Boleij A, Schaeps RM, Tjalsma H. Association between Streptococcus bovis and colon cancer. J Clin Microbiol. 2009;47:516.
93. Nagamine CM, Sohn JJ, Rickman BH, Rogers AB, Fox JG, Schauer DB. Helicobacter hepaticus infection promotes colon tumorigenesis in the BALB/c-Rag2(–/–) Apc(Min/+) mouse. Infect Immun. 2008;76:2758-66.
94. Pellicano R, Ménard A, Rizzetto M, Mégraud F. Helicobacter species and liver diseases: association or causation?. Lancet Infect Dis. 2008;8:254-60.
95. Steinberg D, Naggar CZ. Streptococcus bovis endocarditis with carcinoma of the colon. N Engl J Med. 1977;297:1354-5.
96. Corredoira J, Alonso MP, Coira A, Varela J. Association between Streptococcus infantarius (Formerly S. bovis II/1) Bacteremia and Noncolonic Cancer. J Clin Microbiol. 2008;46:1570.
97. Abdulamir AS, Hafidh RR, Mahdi LK, Al-jeboori T, Abubaker F. Investigation into the controversial association of Streptococcus gallolyticus with colorectal cancer and adenoma. BMC Cancer. 2009;9:403.
98. Ferrari A, Botrugno I, Bombelli E, Dominioni T, Cavazzi E, Dionigi P. Colonoscopy is mandatory after Streptococcus bovis endocarditis: a lesson still not learned. Case report. World J Surg Oncol. 2008;6:49.
99. Schneider D, Liaw L, Daniel C, Athanasopoulos AN, Herrmann M, Preissner KT, et al. Inhibition of breast cancer cell adhesion and bone metastasis by the extracellular adherence protein of Staphylococcus aureus. Biochem Biophys Res Commun. 2007;357:282-8.
100. Edey AJ, Bentley PG, Garrett JP, Liebmann RD. Ductal breast carcinoma presenting with methicillin-resistant Staphylococcus aureus mastitis. Breast J. 2005;11:491-2.
101. Ma Z, Liu L, Zhang F, Yu M, Wang K, Luo J, et al. Human papillomavirus type 16 exists in bacteria isolated from cervical cancer biopsies. J Int Med Res. 2009;37:1065-74.
102. Gugger M, Reubi JC. Gastrin-releasing peptide receptors in non-neoplastic and neoplastic human breast. Am J Pathol. 1999;155:2067-76.
103. Yonemori K, Sumi M, Fujimoto N, Ito Y, Imai A, Kagami Y, et al. Progastrin-releasing peptide as a factor predicting the incidence of brain metastasis in patients with small cell lung carcinoma with limited disease receiving prophylactic cranial irradiation. Cancer. 2005;104:811-6.
104. Prelipcean CC, Mihai C, Gogalniceanu P, Mitrica D, Drug VL, Stanciu C. Extragastric manifestations of Helicobacter pylori infection. Rev Med Chir Soc Med Nat Iasi. 2007;111:575-83.
105. Theoharides TC. Mast cells and pancreatic cancer. N Engl J Med. 2008;358:1860-1.
106. Kountouras J, Zavos C, Diamantidis MD, Deretzi G, Grigoriadis N, Tsapournas G, et al. A concept of Helicobacter pylori and stress-secreted mast cells' potential involvement in brain metastases. J Neuroimmunol. 2009;209:121-2.
107. Leverkus M, Finner AM, Pokrywka A, Franke I, Gollnick H. Metastatic squamous cell carcinoma of the ankle in long-standing untreated acrodermatitis chronica atrophicans. Dermatology. 2008;217:215-8.
108. Verma V, Shen D, Sieving PC, Chan CC. The role of infectious agents in the etiology of ocular adnexal neoplasia. Surv Ophthalmol. 2008;53:312-31.
109. Chan CC, Shen D, Mochizuki M, Gonzales JA, Yuen HK, Guex-Crosier Y, et al. Detection of Helicobacter pylori and Chlamydia pneumoniae genes in primary orbital lymphoma. Trans Am Ophthalmol Soc. 2006;104:62-70.
110. Lee SB, Yang JW, Kim CS. The association between conjunctival MALT lymphoma and Helicobacter pylori. Br J Ophthalmol. 2008;92:534-6.
111. Chanudet E, Zhou Y, Bacon CM, Wotherspoon AC, Müller-Hermelink HK, Adam P, et al. Chlamydia psittaci is variably associated with ocular adnexal MALT lymphoma in different geographical regions. J Pathol. 2006;209:344-51.
112. Aigelsreiter A, Leitner E, Deutsch AJ, Kessler HH, Stelzl E, Beham-Schmid C, et al. Chlamydia psittaci in MALT lymphomas of ocular adnexals: the Austrian experience. Leuk Res. 2008;32:1292-4.
113. Vargas RL, Fallone E, Felgar RE, Friedberg JW, Arbini AA, Andersen AA, et al. Is there an association between ocular adnexal lymphoma and infection with Chlamydia psittaci? The University of Rochester experience. Leuk Res. 2006;30:547-51.
114. Liu YC, Ohyashiki JH, Ito Y, Iwaya K, Serizawa H, Mukai K, et al. Chlamydia psittaci in ocular adnexal lymphoma: Japanese experience. Leuk Res. 2006;30:1587-9.
115. Quint KD, de Koning MN, Geraets DT, Quint WG, Pirog EC. Comprehensive analysis of human papillomavirus and Chlamydia trachomatis in in-situ and invasive cervical adenocarcinoma. Gynecol Oncol. 2009;114:390-4.
116. Carvalho JP, Carvalho FM. Is Chlamydia-infected tubal fimbria the origin of ovarian cancer? Med Hypotheses. 2008;71:690-3.
117. Xu Y, Stange-Thomann N, Weber G, Bo R, Dodge S, David RG, et al. Pathogen discovery from human tissue by sequence-based computational subtraction. Genomics. 2003;81:329-35. Errata in: Genomics. 2003;81:648.
118. Feng H, Taylor JL, Benos PV, Newton R, Waddell K, Lucas SB, et al. Human transcriptome subtraction by using short sequence tags to search for tumor viruses in conjunctival carcinoma. J Virol. 2007;81:11332-40.
119. Duncan CG, Leary RJ, Lin JC, Cummins J, Di C, Schaefer CF, et al. Identification of microbial DNA in human cancer. BMC Med Genomics. 2009;8:2-22.
120. Sleator RD, Shortall C, Hill C. Metagenomics. Lett Appl Microbiol. 2008;47:361-6.
121. Ansorge WJ. Next-generation DNA sequencing techniques. N Biotechnol. 2009;25:195-203.
122. Avila M, Ojcius DM, Yilmaz O. The oral microbiota: living with a permanent guest. DNA Cell Biol. 2009;28:405-11.
123. Ventura M, Turroni F, Canchaya C, Vaughan EE, O'Toole PW, Van Sinderen D. Microbial diversity in the human intestine and novel insights from metagenomics. Front Biosci. 2009;14:3214-21.
124. Muyzer G, De Waal EC, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol. 1993;59:695-700.
125. Schwieger F, Tebbe CC. A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. Appl Environ Microbiol. 1998;64:4870-6.
126. Peix A, Rivas R, Velázquez E, Mateos PF, Martínez-Molina E, Muñoz-Herrera A, et al. Application of horizontal staircase electrophoresis in agarose minigels to the random intergenic spacer analysis of clinical samples. Electrophoresis. 2005;26:4402-10.
127. Scanlan PD, Shanahan F, Marchesi JR. Culture-independent analysis of desulfovibrios in the human distal colon of

healthy, colorectal cancer and polypectomized individuals. FEMS Microbiol Ecol. 2009;69:213-21.
128. Lampe JW. The Human Microbiome Project: Getting to the guts of the matter in cancer epidemiology. Cancer Epidemiol Biomarkers Prev. 2008;17:2523-32.
129. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. Gastroenterology. 2009;137: 588-97.
130. Van Vliet MJ, Tissing WJ, Dun CA, Meessen NE, Kamps WA, De Bont ES, et al. Chemotherapy treatment in pediatric patients with acute myeloid leukemia receiving antimicrobial prophylaxis leads to a relative increase of colonization with potentially pathogenic bacteria in the gut. Clin Infect Dis. 2009;49:262-70.
131. Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS ONE. 2010;5:e9836.
132. Van Vliet MJ, Harmsen HJ, De Bont ES, Tissing WJ. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. PLoS Pathog. 2010;6:e1000879.