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MICROORGANISMS AND CANCER

JOSEF BEUTH

Institute of Naturopathy, Medical Faculty, University of Cologne,
Cologne, Germany

SUMMARY

It is well established that defined microorganisms may cause cancer. Basic, molecular mechanisms are known and might contribute to innovative prophylaxis or treatment strategies, e.g. vaccination.

The ability of defined attenuated microorganisms to cause tumour regression is well known, however, the mode of action mostly remained unknown. It is generally believed that e.g. bacteria (or their products, components; probiotics) activate the immune system and that the activated immune system is responsible for cancer regression. However, experiments with *Toxoplasma gondii* have shown that the inhibition of angiogenesis may also contribute to cancer regression, at least in the case of *T. gondii*. Furthermore, the proliferation of anaerobic bacteria in the core of tumours is well established. However, there is a lack of knowledge whether local anti-tumourous anaerobic bacteria secrete soluble factors that could induce cancer cell death.

Administration of bacterial probiotics in defined stages of malignant disease and its treatment is a promising option in complementary oncology and was shown to restore immune functions and to decrease amount and severity of side effects of tumour destructive standard treatments.

MICROORGANISMS: CAUSATIVE AGENTS FOR MALIGNANT DISEASE

Distinct microorganisms are known to cause malignant diseases (cancer), amongst them *Helicobacter pylori* (gastric cancer), human papilloma virus, HPV (cervical cancer), hepatitis B/C virus, HBV/HCV (hepatocellular carcinoma), Epstein-Barr virus, EBV (lymphoproliferative diseases), *Schistosoma haematobium* (urinary bladder cancer) (Table 1). Basic mechanisms of microbial invasion, intracellular uptake and tumourigenicity are well known and confirmed for the microorganisms mentioned (*Pfister*, 2001), however, an array of further microorganisms are suspected to be involved in malignant, metabolic, and autoimmune diseases. Future investigations have to focus on the provoking question: *Are all diseases infectious?* So far, scientific evidence of microorganisms being causative agents for malignant diseases is limited to defined species and warrants further interdisciplinary investigations.

Table 1: Microorganisms: Aetiologic agents for cancer

Aetiology definitely demonstrated:
H. pylori gastric cancer
S. haematobium urinary bladder cancer
EBV nasopharynx cancer; Burkitt's and immunoblastic B-cell lymphoma
HPV (Type 16,18) cervical cancer
HBV/HCV hepatocellular carcinoma
Aetiology supposed:
HPV (Type 5,8,15) non-melanoma skin cancer
HPV (Type 16,18) larynx-, penis-, vulva-cancer/carcinoma
EBV M. Hodgkin
HHV (Type 8) Kaposi sarcoma

MICROORGANISMS: TREATMENT STRATEGY FOR MALIGNANT DISEASE

Administration of microorganisms (mainly bacteria or their products/extracts; sometimes viral or fungal agents or components) in the treatment of cancer is less widely known in the scientific community. It goes back more than 100 years when William B. Coley, physician and surgeon of the Memorial (Sloan-Kettering) Hospital, New York, observed that many of his cancer patients showed tumour regression when they were infected with bacterial pathogens. Treatment to eliminate the infections resulted in cancer relapse (*Coley*, 1893). He developed a treatment modality by making extracts of defined bacteria (e.g. *Streptococcus pyogenes, Serratia marcescens*) called *Coley's Toxin* which he administered to shrink tumours in his patients (*Coley*, 1893; *Nauts* et al., 1946).

Subsequently, other bacteria have been investigated in an effort to reduce the growth or the size of tumours. The most prominent example would be the use of *Mycobacterium bovis* BCG (Bacillus Calmette-Guerin) the vaccine strain in the treatment of defined stages

Table 2: Evidence-based medicine: Basis for clinical evaluation (Centre of Evidence-Based Medicine, University of Oxford, UK)

Levels of evidence:	
Ia:	Metane alysis of RCTs
Ib:	RCT
IIa:	Metane alysis of epidemiological/cohort studies
IIb:	Epidemiological/cohort study
III:	Non-randomised/experimental study
IV:	Case report
V:	Expert opinion/consensus

Table 3: EBM-Evaluation of microorganisms for cancer treatment

M. bovis BCG urinary bladder cancer EBM level Ib
P. avidum KP-40 colorectal carcinoma EBM level Ib
Coley's Toxin diverse cancers, sarcomas EBM level III
Newcastle Disease Virus lymphoproliferative diseases EBM level III
S. pyogenes OK 432 diverse cancers EBM level III
C. parvum diverse cancers EBM level III

of urinary bladder cancer (*Lamm* et al., 1980). Several good clinical practice (GCP) performed clinical studies (Randomised Controlled Trials; RCTs) have shown a clear relationship between the use of *M. bovis* BCG immunoprophylaxis after surgical removal of the tumour and the decreased recurrence rate or the prolonged relapse-free interval (*Lamm* et al., 1980; *Morales* et al., 1976).

Currently, *M. bovis* BCG immunoprophylaxis is evidence-based in agreement with proposals of the Centre of Evidence-Based Medicine (EBM), University of Oxford, UK (Table 2), since EBM-levels Ib and IIb studies are available (*Agarwala* and *Kirkwood*, 1998; *Sheperd*, 1997). However, long-term administration of BCG might induce problems, e.g. lack of predictability of its effectiveness and serious side effects like sepsis leading to the death of patients (*O'Donnell* and *DeWolf*, 1995).
The mode of action of BCG to induce its antineoplastic effect (see Table 3) is suggested to result from its effects on the (local, mucosal) immune system, with mononuclear cells (T-lymphocytes, monocytes) playing a major role (*Ratlift* et al., 1993). Thus, intravesical instillation of BCG induces a non-specific cystitis, which is accompanied by local production of cytokines and accumulation of inflammatory cells being able to damage malignant cells (*Alexandroff* et al., 1999). The requirement of live cells of BCG for its anticancer activity is reflected in the fact that monocytes and helper T-lymphocytes type 1 (TH_1) are most important for its effectiveness (*Thanhauser* et al., 1995) and that high doses of defined vitamins have shown a positive effect on the treatment of bladder cancer in human clinical trials (*Lamm* et al., 1994).
The ability of bacteria to modulate the immune response to non-related antigens is well documented. *Propionibacterium* species are amongst the most potent immunomodulators stimulating cell populations involved in non-specific

Table 4: Hypotheses on basic mechanisms:
Microorganisms as treatment strategies for malignant diseases

Local application: → Coley's Toxin; *S. pyogenes* OK 432; *M. bovis* BCG
• Microbial toxin lyses cancer cells
• Inflammation activates cytokines, immune cells
• Immunoactivation (cytokines, immune cells)
Systemic application: → Coley's Toxin; *P. avidum* KP-40; *C. parvum*; *S. pyogenes* OK 432
• Inflammation
• Immunoactivation
• Fever induction
→ all induce cytokine release; immune cell activation

Table 5: History of immunomodulating *Propionibacterium* species

Basis: Promising data on bacterial immunomodulators
→ Coley's Toxin; *M. bovis* BCG
1980: Selection of *P. avidum* KP-40
→ optimum immunomodulator from about 200 strains
1981: Basic investigations on immunomodulating effects
1982: Clinical studies in oncology, infectiology
1997: Research on active components
→ LTA, glycopeptides
2001: Research on oral application of *P. avidum* KP-40

resistance (*Jeljaszewicz* et al., 1982; *Isenberg* et al., 1995). Three species (*Propionibacterium acnes, P. granulosum, P. avidum*) appeared to be of special medical interest and after evaluating the immunoactive potential of a great number of strains (*Ko* et al., 1981) *P. avidum* KP-40 and *P. granulosum* KP-45 were selected for further experimental and clinical studies. For practical reasons (e.g. cultivation procedure, biological and immunological standardisation) *P. avidum* KP-40 was preferably introduced for clinical evaluation, although its immunoactive capacity is absolutely identical to *P. granulosum* KP-45.

The obvious therapeutical benefit of *P. avidum* KP-40 treatment in neoplastic disease induced a great amount of experimental studies (*Isenberg* et al., 1995; *Pulverer* et al., 1985). During these investigations we were able to determine the effects of *P. avidum* KP-40 on thymocyte proliferation, maturation and emigration into peripheral blood using a murine model. Single intraperitoneal administration of the optimal immunomodulating dose of *P. avidum* KP-40 (1 mg per mouse, as determined in preceding studies) to BALB/c-mice resulted in enhanced thymus weight and accelerated thymocyte maturation (generally leading to emigration of these cells into peripheral blood), followed by enhanced proliferation of immature cells. Furthermore we found that absolute counts of peripheral blood lymphocytes (PBL) and monocytes (PBM) were significantly enhanced as well as the expression of activation markers (e.g. interleukin (IL)-2 receptors on PBL; MAC-3 antigens on PBM) with peak values 6 days after *P. avidum* KP-40 injection (*Isenberg* et al., 1995; *Beuth* et al., 1990).

Table 6: Preclinical evaluation of P. avidum KP-40

• Proliferation/activation of RES cells
→ spleen cells, macrophages, monocytes
• Proliferation, maturation and emigration of thymocytes
• Activation of peripheral blood cells
→ granulocytes, monocytes, lymphocytes, NK-cells
• Anti-infective activities
→ against bacteria, viruses, parasites
• Antineoplastic/antimetastatic activities
→ in various murine models

To evaluate antitumour/antimetastatic effects of *P. avidum* KP-40 induced immunomodulation, BALB/-c mice were intravenously challenged with RAW 117-H lymphosarcoma cells and checked for liver tumour colonisation as described elsewhere (*Beuth* et al., 1987). Compared to a control group the number of liver colonies was significantly lower in *P. avidum* KP-40 treated mice (*Isenberg* et al., 1995; *Beuth* et al., 1987).

Clinical investigations proved that surgical treatment of malignant diseases (e.g. colorectal carcinoma) induced an evident decrease of peripheral blood lymphocyte counts and activity, as compared to pre-operative values. However, single pre-operative administration of *P. avidum* KP-40 induced a considerable increase of peripheral blood cell counts and activities, especially of lymphocytes. Clinical effects of pre-operative immunostimulation by propionibacteria were investigated in prospectively randomised clinical studies in colorectal carcinoma patients. Beneficial effects of survival time, local tumour recurrence and distant metastasis could be demonstrated in stages I and II, whereas no advantage of immunotherapy was found in advanced stages III and IV (14).

Another prospectively randomised clinical study was initiated on the quality of life of colorectal carcinoma patients. Three months after surgical treatment negative effects could not be determined after immunotherapy. Quality of life even proved to be better in patients with abdomino-perineal resection as compared to non *P. avidum* KP-40 treated control patients (*Isenberg* et al., 1995).

Generally, the activated immune system provides protection from infectious pathogens and growth/spread of malignant cells through mechanisms of recognition and elimination. Accordingly, Coley's Toxin, *M. bovis* and *P. avidum* KP-40 could be shown to be effective in the treatment of neoplastic disease in human medicine. Further clinical studies are warranted to confirm and enlarge these promising data.

MICROORGANISMS/PROBIOTICS: INNOVATIVE COMPLEMENTARY TREATMENT IN ONCOLOGY

New approaches to curative cancer therapy are being explored and evaluated around the world. Great hopes have been placed in the *Human Genome Project* as well as in advances in the fields of molecular biology, molecular genetics and immunology, without leading to the validation of a new beneficial concept of treatment.

Searching for new substances of therapeutic importance (e.g. in rain forests, oceans or by developing new technologies) seems promising. However, it is time-consuming and expensive because scientific evaluation is an obligate prerequisite before clinical application. Currently, the optimisation of curative cancer therapy seems to be possible especially through the development of interdisciplinary concepts.

In the United States, the use of tumour-destructive standard therapies (surgery, chemotherapy, radiotherapy) did not significantly lower cancer mortality over the last 20 years. Despite extensive efforts in both research and therapy in response to *President Nixon's declaration of war against cancer* at the beginning of the 1970's, age-adjusted cancer mortality even increased about 6% (*Bailar* and *Gornik*, 1997). Therapeutically beneficial results were achieved for relatively rare types of tumours such as lymphoma, leukaemia, testicular tumours. This outcome demanded innovative concepts of treatment and ushered in the scientific, experimental, and clinical efficacy testing of

Table 7: Scientifically-based complementary cancer treatment

- ***No alternative*** to standard treatment, but ***optimisation***
- Evaluated in RCTs, thus ***integrated in EBM***
- Integrated in Disease Management Program; DMP in Germany
- Accepted by German Medical Association and Health Insurance Companies
- Integrated into educational curricula for German physicians
- ***Rigorously demanded by patients***

therapeutic approaches used in complementary oncology (*Abel*, 1995).

About 80% of all cancer patients in Germany use complementary medicine, often without knowledge of the attending oncologists (*Beuth*, 2002). Their main motives are

- to actively participate in fighting against the disease or promoting recovery,
- to activate the immune system,
- to optimise standard therapy.

These understandable wishes need to be addressed with critical openness, and therapists should be aware that active patients profit from the activation of their psycho-neuro-immunological system.

As per definition, therapeutic approaches of complementary oncology do not replace the approved standard therapies. Hence, they are not *alternative therapies*. Complementary approaches in oncology proved to be beneficial additions to the tumour destructive standard therapies to optimise them (*Beuth*, 2002).

Preliminary data from scientifically-based studies have demonstrated the importance of various approaches. The benefits to the patients included improvement of the quality of life, reduction of symptoms and side effects due to standard therapy, and improvement of the immunological state (*Beuth*, 2002).

Indication-based administration of probiotics (live/attenuated/killed micro-organisms, their components or metabolic products) is part of the scientifically-based complementary oncology. Indications include peri-/post-tumour-destructive immunocompromisation; after care-/regeneration period and its accompanying weakness/fatigue; disease-/treatment-induced (metabolic) disorders, e.g. in the gastro-intestinal (GI)-tract and regeneration of physiological microflora (*Beuth*, 2002). So far, EBM-level III/IV studies are available and show beneficial effects (safety and efficacy) of the indication-based administration of *medical probiotics*.

Table 8: Scientifically-based complementary medicine

- Recommended EBM-level I/II evaluated therapies:
  - → Nutrition optimisation/guidance
  - → Moderate sportive activities
  - → Psycho-oncological guidance
  - → Sodium selenite (on indication)
  - → Standardised proteolytic enzymes (on indication)
  - → Immunoactivating standardised mistletoe extract (on indication)
- Extended, EBM-level III/IV evaluated therapies:
  - → E.g. probiotic/microbiological treatment

Table 9: Beneficial effects of medical probiotics in cancer treatment

• Lactic acid producing bacteria/probiotics → e.g. *Lactobacillus* species; *Bifidobacterium* species
• Bacteria/probiotics of GI-tract origin → e.g. *E. coli; E. faecalis*
⇒ Regulate metabolism (→ e.g. GI-tract) ⇒ Optimise physiological microflora ⇒ Modulate immune functions (→ e.g. MALT)

However, further studies (EBM-level I or II) are warranted as to integrate this complementary medication into standard treatment concepts.

The lack of predictability of efficacy, the non-specific (non-controlled) immune response and the associated side-effects and toxicity have so far limited the use of live/attenuated/killed microorganisms and their components/products in the treatment of cancer. Innovative approaches to tailor specific molecules to target defined cells and their metabolism might lead to a specific treatment modality for malignant diseases. Current investigations are promising.

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