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Bacteria as tumor therapeutics?

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Addendum to: Stritzker, et al. Myristoylation negative msbB-mutants of probiotic ***E. coli*** Nissle 1917 retain tumor specific colonization properties but show less side effects in immunocompetent mice. Biobugs 2010; In this issue.

Already about 150 years ago the physicians W. Busch and F. Fehleisen noticed regression of tumors in cancer patients after accidental infections by erysipelas. In 1868, Busch was the first who intentionally inoculated a cancer patient with erysipelas and he noticed shrinkage of the malignancy.[1] Fehleisen repeated this treatment in 1882 and eventually identified *Streptococcus pyogenes* as the causative agent of erysipelas.[2] The American surgeon W.B. Coley systematically treated bone and soft tissue sarcoma patients first with live Streptococcus and after the death of 2 of 3 patients caused by the Streptococcus infection he used heat-killed streptococcal organisms combined with heat-killed *Serratia marcescens*. This concoction became known as Coley's Toxin. He reported a rather high success rate in treating patients with sarcomas with "Coley's Toxin."[3] However, his studies were controversial, because they were anecdotal and difficult to repeat.

Subsequently, it has been realized that anaerobic bacteria can selectively grow in tumors. However, these bacteria were not suitable for cancer therapy because of their high pathogenicity. Later on studies in animal models revealed that obligate anaerobic bacteria such as clostridia species proliferate preferentially in necrotic and therefore anaerobic regions of solid tumors. This actually resulted in tumor regression but was accompanied by acute toxicity and most animals became ill or died. Application of non-pathogenic Clostridium strains did not cause significant tumor regression.[4] Despite these discouraging results researchers have screened several obligate and facultative anaerobic bacterial genera for their ability to accumulate in tumors in animal models. In mice Clostridium, Salmonella, Bifidobacterium and Escherichia specifically accumulate in tumors over normal tissue. The targeting of Clostridium to the tumor is the result of the spores to germinate selectively under anaerobic conditions found in the tumor.[5] *Clostridium novyi* depleted of its lethal toxin gene (*C. novyi*-NT) in combination with a single dose of liposomal doxorubicin led to the eradication of large, established tumors in mice.[6] A general problem with Clostridium e.g., of the non-pathogenic *C. butyricum* strain M-55 is that although they are able to induce liquefaction of the tumor (oncolysis), there remains always a well oxygenated outer rim of the tumor leading to regrowth. In one clinical trial with *C. butyricum* M-55 spores for treatment of glioblastomas liquefaction of tumors was achieved. Unfortunately, the rate of recurrence, however, remained uninfluenced and none of the patients showed improvement in their life span.[7,8]

Intratumoral targeting in animal models could be enhanced for *Salmonella typhimurium* by knocking out the gene for the ribose/galactose receptor causing in addition apoptosis in cancer cells.[9] In another approach, *S. typhimurium* was made auxo trophic for the amino acids arginine and leucine. These mutations preclude growth in normal tissue but do not reduce bacterial virulence in cancer cells. This auxo trophic strain administered to nude mice with human pancreatic cancer and fibrosarcoma experimental metastases eradicated these metastases after 7–21 days of treatment without the need of chemo therapy or any other treatment. Interestingly, no adverse effects were reported.[10] In contrast, in clinical trials the attenuated *purI*- and *msbB*-deletion mutant of *S. typhimurium* strain VNP20009 was

employed. This strain relies on an external source for adenine because of the deleted *purI* gene. It is also less toxic due to the *msbB* deletion resulting in lower TNFα induction, subsequently reducing the risk for septic shock. However, no patient having received VNP20009 exhibited tumor regression and only three patients had tumors containing viable bacteria.[11] Obviously, conditions for tumor colonization in rodents and humans differ.

The only established cancer therapy employing bacteria is the one making use of Bacillus Calmette-Guérin (BCG). This attenuated *Mycobacterium bovis* strain was successfully used by Morales, Eidinger and Bruce in 1976 for the treatment of superficial bladder cancer.[12] Today BCG has become the treatment of choice for high risk, superficial bladder cancer in most countries, with an increasing rate of treating approximately one million patients per year.[13]

Only recently the use of probiotic instead of pathogenic or virulence attenuated strains was introduced into this field. Maybe the best characterized probiotic is the *E. coli* strain Nissle 1917 (EcN), which is also licensed as a pharmaceutical in several countries for the treatment of diseases affecting the digestive tract such as diarrhea and colitis ulcerosa.[14] It seems also to be very safe after oral application taking into account its use as a drug for several decades without any reports of serious side effects, and it's lack of virulence genes.[15] Another safety feature of EcN distinguishing it from probiotic lactobacilli is its serum sensitivity caused by EcN's semi-rough LPS. This in turn results from a point mutation in the *wzy* gene encoding the O-antigen polymerase.[16] EcN in contrast to *S. typhimurium* has not to be genetically altered to achieve a very high selectivity for tumor colonization in mice. In fact, EcN did not colonize liver and spleen at all and even almost exclusively the tumor tissue, even in immunocompromised animals.[17] These properties make EcN an appealing candidate for approaches to detect and destroy solid tumors by colonization with bacteria. Furthermore, remote controlled gene expression in recombinant EcN strains in live mice was achieved. The expression of genes of interest could be induced by oral or systemic application of the inducer substance L-arabinose, L-rhamnose or anhydrotetracyclin.[17,18] These constructs lay the foundation for recombinant EcN able to deliver a therapeutic substance, e.g. an enzyme for transformation of a prodrug into a drug specifically in the tumor and only after induction of the gene encoding the enzyme by oral administration of a simple substance. Such an approach must be adopted since EcN has so far not shown any intrinsic anti-tumor activity in spite of efficient tumor colonization. But the problem of probably severe immune stimulation by lipid A, as for all Gram-negative bacteria, remained. An important step forward on the road to an even safer derivate of EcN suitable for intra venous application was accomplished by the group of Szalay reported in this issue of *Bioengineered Bugs*. They constructed an *msbB* mutant of EcN, which no longer is able to myristoylate the lipid A in this strain. As a consequence, the resulting strain was less toxic for immunocompetent mice but still specifically colonized tumors. For *E. coli* K-12 *msbB* mutants, a dramatically reduced bioactivity of the mainly penta-acylated LPS had been reported earlier supporting the observations published in this paper.[19,20] The combination of the latest progress, i.e., remote controlled gene expression in the *msbB* mutant should be the next step taken on the way to design an even safer EcN strain able to deliver therapeutic molecules in a highly specific spatial and temporal fashion suitable for bacteria mediated anti-cancer therapy.

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