1 Introduction

Many cases of cancer can now be cured by surgery, radiation and chemotherapy. However this treatment has its limitations. Hope comes from new methods directed against single steps of tumorigenesis which will be even more effective. The available chemotherapeutic drugs often fail to cure patients because they kill many healthy cells und thus bring on serious side effects that limit the doses physicians can administer.

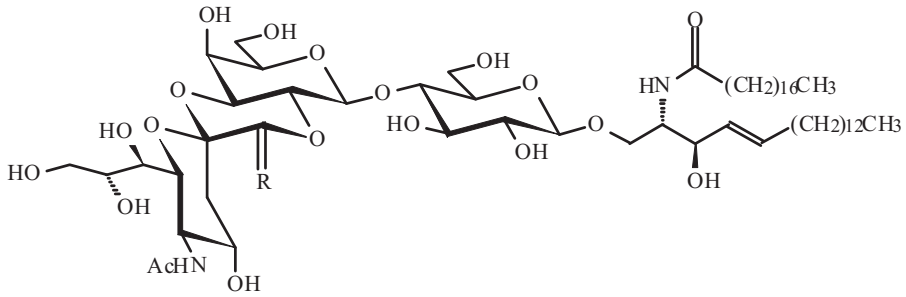
A simple, universal treatment that is effective for all cancers, is extremely unlikely to emerge anytime in the near future. But a large set of more specific and less toxic treatments is probably nearer at hand.

The conversion of normal cells into invasive cancers with metastatic potential is a process that involves several steps. These steps are manifested in distinguishable histological and temporal stages (normal tissue, hyperplasia with a high incidence of proliferating cells, dysplasia with the induction of angiogenesis before the emergence of frank tumors with metastatic potential). Analysis of the later stages of tumor progression has resulted in a multi-step theory of tumorigenesis on the basis of genetic changes involving activation of oncogenes, inactivation of tumor suppressor genes, and altered expression of tumor-associated molecules. Whereas cancer research has historically focussed on such intrinsic events, it has become evident that extrinsic factors – the local stromal microenvironment - also regulate critical parameters of tumorigenesis and evolve and undergo multi-step reconstruction paralleling neoplastic progression. Thus, the stroma, and its resident reactive host cells, constitutes a second important component of solid tumors.[1]

1.1. Regulation of EGF receptor activity by HK1-ceramide, a stable synthesized analogue of the ganglioside GM3-lactone

The involvement of gangliosides in tumor development and progression, e.g. processes of cell proliferation, migration and adhesion has been widely described.[2,3] In addition to proteins and lipids, carbohydrates - of which gangliosides constitute a large part - are essential elements of the cell surface. Gangliosides are neuraminic acid containing glycosphingolipids which are responsible for a variety of biological recognition processes. They are anchored in the lipid bilayer of the membrane by the ceramide portion with the carbohydrate moiety being exposed on the outside of the cell. The ganglioside pattern on the surface of tumor cells is distinguished from normal cells, for instance, the amount of GM3, GM2 and GD3 has been found to be increased on malignant melanomas. [4,5] It has been suggested that an equilibrium exists between the gangliosides and their lactones e.g. GM3 and its lactone **1** (Fig. 1) by reaction of the sialic acid moiety with a hydroxyl group of an adjacent sugar in the molecule. The ganglioside-lactones are thought to be formed to a higher extent on malignant cells probably due to a lower pH on these cells and may therefore play an important role as tumor associated antigens. [6]

Figure 1



Structure of GM3-lactone and HK1-ceramide. GM3-lactone **1**: R = O. HK1-ceramid **2**: R = H_2

It has also been demonstrated that the GM3-lactone is more immunogenic in comparison to GM3. The anti-melanoma GM3 antibody, M2590, has shown a high affinity for GM3-lactone but a low affinity for GM3.[7] Ganglioside-lactones have become more of interest, particularly GM3-lactone, since it has been identified in mammary gland tumors and gastric tumors [8] and was found to bind to influenza virus hemagglutinin [9]. Furthermore, the gangliosides extracted from mullet milt were determined to be GM3, GM3 lactone, GM3 methylester, and 9-O-acetyl GM3.[10]

Recently, Tietze et al. succeeded in the preparation of the GM3-lactone analogue HK1-ceramide **2** (Fig. 1), which contains an ether moiety instead of the lactone functionality and is stable under physiological conditions.[11] To our knowledge HK1-ceramide **2** is the first and the only stable ganglioside-lactone-analogue so far synthesized. Therefore, HK1-ceramide seems to be a perfect tool to analyze the mode of action of the hypothetically fixed GM3-lactone or GM3-lactone-like conformation.

Ganglioside GM3 has been described to modulate cell growth through inhibition of EGF receptor associated tyrosine kinase.[12] Upon binding of EGF to the extracellular domain of the EGF receptor the tyrosine kinase is activated leading to the tyrosine phosphorylation of intracellular substrates such as Grb2, sos, ras, raf, MEK, ERK and ELK-1.[13] The effect of GM3 appears to be mediated by direct inhibition of EGF-receptor autophosphorylation and dimerization [12,14] and not by acting directly on the intracellular intermediates of EGF receptor signaling.[15]

The goal of this study was to determine if the lactone form of GM3 has a similar inhibitory effect on EGF induced cell growth and EGF-R tyrosine phosphorylation. In order to accomplish this, the influence of the stable ganglioside-lactone-analogue

HK-1 ceramide toward EGF receptor signaling and EGF mediated cell growth was examined and directly compared to the effects of GM3.

1.2. A novel polyclonal antibody directed against the lactone form of ganglioside GM3: Analyses of its potential as tumor marker for melanomas.

Melanoma is a tumor of the melanocytes, the melanin producing cells, distributed in the skin as well as in other organs. The prominent features of the melanocytes, in addition to their unique ability to synthesize melanin, is their motility in early life. Melanocytes express gangliosides, the qualitative pattern of which is somewhat characteristic of other extra-neural tissues. The quantity of the gangliosides is higher than that of other extra-neutral tissues. GM3 is the most predominant ganglioside. Other gangliosides of the melanocytes, which include GD3, GM2, GD1a, and GT1b, constitute less than 10 % of the total.[16]

The onset of the neoplastic transformation of melanocytes triggers the enzyme machinery associated with glycosylation, particularly that related to the addition of sialic acid (Sia) and N-acetyl galactosamine (Ga1Nac). Adding sialic acid to the preexisting sialic acid of GM3 results in the formation of GD3 [16] and GD3 accumulation is an important event associated with the growth and proliferation of melanoma.[17] Since the differences in the ganglioside profile of neoplastically transformed melanocytes correlates with the changes in the functional properties of the malignant cells, such as proliferation, migration, adhesion to basement membrane components, infiltration and metastasis,17,18 it was of interest to search for differences in the ganglioside pattern expressed in melanoma cells in comparison to naevus cells which comprise benign melanocyte tumors. Previous studies have

shown that it is possible to identify differences in protein antigen expression between benign and malignant melanocytes by searching for monoclonal antibodies which show differential reactivity with these cells *in situ.* [19,20] Mab MacG1 e.g. distinguishes between malignant melanoma and benign melanocytic nevi in tissue sections. Mab MacG1(IgG2a) was obtained following immunization with a mixture of gangliosides prepared from a melanoma lymph node metastasis. But in contrast to other antibodies directed to GM3, MacG1 does not stain the melanoma cells themeselves but rather granules associated with tumor infiltrating macrophages. *In vitro* studies suggest that the MacG1 epitope is generated during phagocytic degradation of ganglioside rich cellular debris. When tested with a panel of purified gangliosides, MacG1 showed a lack of reactivity with GM3-lactone. [21]

Melanoma-shed gangliosides have modulatory influence on both the humoral and cellular immune system in humans. Low concentrations are stimulatory, whereas increasing concentrations lead to a potent inhibition of all lymphocyte functions, this inhibition being reversible by removing the gangliosides from the extracellular medium. The shed gangliosides might thus be involved in the protection of tumor cells from immune killing.

The goal of this study was to investigate the expression pattern of gangliosides on melanoma cells applying the new polyclonal antibody against HK1[48]. It has been shown that this antibody show immunoreactivity with GM3 and its lactone form.