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(71) Applicant *(for all designated States except US)*: MAX-PLANCK GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V. [DE/DE]; Berlin (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: EATON, Suzanne [US/DE]; Reger Str. 2, 01309 Dresden (DE). THIELE, Christoph [DE/DE]; Birkenstr. 32, 01474 Dresden (DE). PANAKOVA, Daniela [SK/DE]; Louisenstr. 12, 01099 Dresden (DE). MAROIS, Eric [FR/DE]; Neubertstr. 27, 01307 Dresden (DE). SPRONG, Hein [NL/NL]; Gerrit Jan van der Veenlaan 11, NL-3705 PE Zeist (NL).

(74) Agent: VOSSIUS & Partner; Siebertstrasse 4, 81675 Munich (DE).

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(54) Title: TREATMENT OF HEDGEHOG- AND WNT-SECRETING TUMORS WITH INHIBITORS OF LIPOPROTEIN PARTICLE BIOGENESIS

(57) Abstract: This invention relates to the use of an inhibitor of Microsomal Triglyceride Transfer Protein (MTP), HMG-CoA reductase, DGAT and/or ACAT for the preparation of a pharmaceutical composition for the treatment of tumors. In a preferred embodiment, growth and/or progression of the tumor are caused by one or more protein of the Wnt or Hedgehog family. Preferred tumors are esophageal tumor, biliary tract tumor, gastric tumor, pancreatic tumor, malignant melanoma, colorectal tumor, squamous cell carcinoma and cervical tumor.

Treatment of Hedgehog- and Wnt-secreting tumors with inhibitors of Lipoprotein particle biogenesis

This invention relates to the use of an inhibitor of Microsomal Triglyceride Transfer Protein (MTP), HMG-CoA reductase, DGAT and/or ACAT for the preparation of a pharmaceutical composition for the treatment of tumors. In a preferred embodiment, growth and/or progression of the tumor are caused by one or more protein of the Wnt or Hedgehog family. Preferred tumors are esophageal tumor, biliary tract tumor, gastric tumor, pancreatic tumor, malignant melanoma, colorectal tumor, squamous cell carcinoma and cervical tumor.

In this specification, a number of documents is cited. The disclosure of these documents, including manufacturer's manuals, is herewith incorporated by reference in its entirety.

Despite intense investigations, the molecular mechanisms leading to tumor formation and cancer are far from being completely understood. Chains of molecular events leading to malignant transformation are emerging, wherein certain mechanisms appear to be generic, while others are specific for certain tumors.

Wnt and Hedgehog family proteins are secreted ligands that play multiple critical roles in the development of multicellular organisms. Alterations in the cellular signaling pathways that respond to these Wnt and Hedgehog family ligands also play causative roles in the initiation and progression of a variety of tumors (Xie and Abbruzzese (2003)). Mutations in Hedgehog signal transduction components give rise to tumors of the skin, muscle and cerebellum.

Alteration in Wingless signal transduction components is a critical step in the development of colon cancer and is associated with a variety of other types of malignancies (reviewed in Giles et al. (2003)). While alterations in the downstream components of these pathways have been known for some time, recent studies have shown that tumorigenesis can depend on the unregulated production of the ligands themselves.

One of the earliest identified oncogenes is Wnt1, whose ectopic activation in mouse mammary cells is the basis for MMTV mediated tumorigenesis (Nusse and Varmus (1992)). Consistent with this, overproduction of a variety of Wnt family ligands has been observed in human breast cancers. Over-expression of Wnt proteins in humans is also symptomatic of many gastric cancers, colorectal cancers, pancreatic cancers, esophageal cancers, squamous cell carcinomas, cervical cancers and malignant melanomas. A causative role has been directly demonstrated for Wnt5a in promoting both cell motility and invasion of malignant melanoma cells (Weeraratna et al. (2002)). Although the extent to which Wnt overproduction contributes to malignant phenotypes is not yet characterized in all cases, the frequency with which mutations in Wnt signaling pathway components promote other neoplasias suggests that Wnt overproduction has tumorigenic consequences.

Overproduction of Hedgehog ligands has been demonstrated to play a direct causal role in promoting growth of tumors of the gastrointestinal tract, including those of the esophagus, stomach biliary tract and pancreas (Berman et al. (2003); Thayer et al. (2003). These tumors are very aggressive and some of the most resistant to current therapy. The relevance of Hedgehog signalling for cancer formation and maintenance has been reviewed in Pasca di Magliano and Hebrok (2003). Preventing secretion of Hedgehog and Wnt proteins should be an effective therapy for cancers that depend on the unregulated production of these ligands.

Paradoxically, Wingless and Hedgehog are covalently modified by lipid, which is thought to mediate their interaction with the exoplasmic face of the plasma membrane. This observation raises perplexing questions about how Wingless and Hedgehog, having affinity for cell membranes, are released from the cells that make them and move through adjacent tissue. As yet, it is unclear how lipid-modified proteins leave the plasma membrane and move over many cell diameters.

The function of lipid modification of Wingless and Hedgehog is not yet understood. In *Drosophila,* mutant Hedgehog proteins that cannot be sterol-modified signal over inappropriately long distances when over-expressed (Porter et al. (1996), Burke et al. (1999)). This would suggest that the role of lipid is to restrict the range of morphogen diffusion through the epithelial plane. On the other hand, mice that harbor this mutant form of Hedgehog in its normal chromosomal context are deficient in long-range Hedgehog signalling (Lewis et al. (2001)). This suggests, in contrast, that lipid

modification may be necessary for movement of the protein. Mutations that prevent the N-terminal palmitoylation of either Wingless or Hedgehog destroy their activity (Chamoun et al. (2001), Lee et al. (2001), Willert et al. (2003)); thus, lipid modification is crucial to the function of these proteins.

In summary, there is evidence for the involvement of proteins of the Wnt or Hedgehog families in tumor formation and progression, however, a significant part of the corresponding molecular events are still obscure, thereby impeding a rational approach to therapy.

The technical problem underlying the present invention was to provide novel means and methods for the treatment of Hedgehog- and/or Wnt-secreting tumors.

Accordingly, this invention relates to the use of an inhibitor of Microsomal Triglyceride Transfer Protein (MTP), HMG-CoA reductase, DGAT and/or ACAT for the preparation of a pharmaceutical composition for the treatment of tumors.

The Microsomal Triglyceride Transfer Protein (MTP) is a heterodimeric lipid transfer protein that catalyzes the transport of triglyceride, cholesteryl ester and phosphatidylcholine between membranes. It is required for assembly and secretion of the lipoproteins containing apolipoprotein B (apoB), i.e., very low density lipoproteins (VLDL) and chylomicrons. VLDL in turn is converted into LDL. Accordingly, inhibition of MTP function would affect the levels of lipoproteins comprising chylomicrons, VLDL and LDL. Similarly, inhibitors of HMG-CoA reductase, DGAT or ACAT (which are enzymes involved in lipid biosynthesis) affect the levels of lipoproteins. The term "inhibitor" designates a compound lowering the activity of a target molecule, preferably by performing one or more of the following effects: (i) the transcription of the gene encoding the protein to be inhibited is lowered, (ii) the translation of the mRNA encoding the protein to be inhibited is lowered, (iii) the protein performs its biochemical function with lowered efficiency in presence of the inhibitor, and (iv) the protein performs its cellular function with lowered efficiency in presence of the inhibitor. In one embodiment, in particular with regard to inhibition of HMG-CoA reductase, the inhibitor is a statin. Compounds falling in class (i) include compounds interfering with the transcriptional machinery and/or its interaction with the promoter of said gene and/or with expression control elements remote from the promoter such as enhancers. Compounds of class (ii) comprise antisense constructs and constructs for performing

RNA interference well known in the art (see, e.g. Zamore (2001) or Tuschl (2001)). Compounds of class (iii) interfere with molecular function of the protein to be inhibited, in case of MTP with its enzymatic activity, in particular with the protein disulfide isomerase activity. Accordingly, active site binding compounds, in particular compounds capable of binding to the active site of any protein disulfide isomerase, are envisaged. More preferred are compounds specifically binding to an active site of MTP. Also envisaged are compounds binding to or blocking substrate binding sites of MTP as are compounds binding to or blocking binding sites of MTP for other interaction partners. An example for such an interaction partner would be apolipoproteinB (apoB). The latter group of compounds blocking binding sites of MTP may be fragments or modified fragments with improved pharmacological properties of the naturally occurring binding partners. Class (iv) includes compounds which do not necessarily directly bind to MTP, but still interfere with MTP activity, for example by binding to and/or inhibiting the function or inhibiting expression of members of a pathway which comprises MTP. These members may be either upstream or downstream of MTP within said pathway.

In a preferred embodiment, the inhibitor is a low molecular weight compound. Low molecular weight compounds are compounds of natural origin or chemically synthesized compounds, preferably with a molecular weight between 100 and 1000, more preferred between 200 and 750, and even more preferred between 300 and 600.

The efficiency of the inhibitor can be quantitized by comparing the level of activity in the presence of the inhibitor to that in the absence of the inhibitor. For example, as an activity measure may be used: the change in amount of mRNA formed, the change in amount of protein formed, the change in amount of substrate converted or product formed, and/or the change in the cellular phenotype or in the phenotype of an organism.

In a preferred embodiment, the level of activity is less than 90%, more preferred less than 80%, 70%, 60% or 50% of the activity in absence of the inhibitor. Yet more preferred are inhibitors lowering the level down to less than 25%, less than 10%, less than 5% or less than 1% of the activity in absence of the inhibitor.

Using Drosophila as a model organism, the inventors have surprisingly shown that Hedgehog and Wingless are released from cells on Lipoprotein particles. It has been

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