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Melanocortin system in cancer-related cachexia

Research Article

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Abstract: The melanocortin system plays a pivotal role in the regulation of appetite and energy balance. It was recognized to play an important role in the development of cancer-related cachexia, a debilitating condition characterized by progressive body wasting associated with anorexia, increased resting energy expediture and loss of fat as well as lean body mass that cannot be simply prevented or treated by adequate nutritional support.

The recent advances in understanding of mechanisms underlying cancer-related cachexia led to consequent recognition of the melanocortin system as an important potential therapeutic target. Several molecules have been made available for animal experiments, including those with oral bioavailability, that act at various checkpoints of the melanocortin system and that might confer singificant benefits for the patients suffering from cancer-related cachexia. The application of melanocortin 4 receptor antagonists/agouti-related peptide agonists has been however restricted to animal models and more pharmacological data will be necessary to progress to clinical trials on humans. Still, pharmacological targeting of the melanocortin system seem to represent an elegant and promising way of treatment of cancer-related cachexia.

Keywords: *Melanocortins • Agouti-related peptide • Cancer • Cachexia*

1. Introduction:

1.1. Cancer cachexia

Cancer cachexia is a devastating metabolic condition characterized by progressive wasting of body weight with simultaneous depletion of skeletal muscles and adipose tissue with or without anorexia [1]. Depending on the tumor type, thirty to eighty percent of those with advanced cancer disease have cachexia and associated symptoms such as anorexia, early satiety, weight loss, weakness, anemia, and edema [2,3]. Generally, patients with pancreatic or gastric cancers have higher incidence of weight loss, while patients with non-Hodgkin's lymphoma, breast cancer, acute nonlymphocytic leukemia, and sarcomas present with the lower frequency of weight loss [4].

Cachexia is a negative prognostic factor in all types of malignancies and is considered the direct cause of more than 20% cancer deaths [2]. With respect to the type of tumor, weight loss occurs in 30–80% of cancer patients and is severe (with weight loss of more than 10% of the pre-cancer body weight) in 15% [3]. Patients with pancreatic or gastric cancer have the highest frequency of cancer-related weight loss, while patients with non-Hodgkin's lymphoma, breast cancer, and acute nonlymphocytic leukemia present with the lowest frequency of weight loss [3] and there is a significant correlation between the weight loss and poor prognosis [4]. Still, even in the types of cancer often associated with cachexia, some patients do not progress into this wasting syndrome. This is possibly due to variations in tumor phenotype or host genetic background, or both,

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that prevents the development of cachexia in some individuals [5].

In the past decade, a substantial progress has been made in understanding the neural networks responsible for the suppresion of appetite often observed in cancer-related cachexia, whereas recognition of melanocortin pathways as well as endocannabinoid system provided important insight into the issue [6] .

By definition, cachexia is characterized by progressive loss of lean body mass (LBM), whereas in starvation LBM is generally preserved to some extent until near death [7], the prognostic effect of weight loss being more pronounced in cancer types with generally favourable prognosis. The non-muscle protein compartment is also generally well preserved, thus distinguishing cachexia from simple starvation [8].

The pathophysiology of cancer cachexia is multifactorial. The loss of fat stores cannot be explained by a reduced apetite as the loss of fat deposits often precedes the onset of anorexia [9] and also increased metabolic rate at rest is observed in cachexia [10]. Cancer cachexia results from the complex tumor-host interactions [11] that cause imbalance favouring catabolism over anabolism in the periphery. The exact nature of underlying mechanisms involving numerous cytokines and adipokines is, however, uncertain. Some evidence has accumulated supporting a hypothesis of cancer cachexia as a distinct metabolic condition – 1) cancer cachexia can be observed in the absence of anorexia and 2) adequate nutritional support does not restitute LBM in the patients with cancer cachexia [12], suggesting that progressive metabolic wasting is not simply due to reduced energy intake. Therefore, there is a consensus that cancer cachexia is a consequence of impaired central as well as peripheral singalling that controls not only appetite but also the energy metabolism of peripheral tissues.

2. Melanocortin system

Multiple diverse processes with distinct pathophysiology, such as renal failure, cardiac failure, cancer, or acquired immunodeficiency syndrome result in most of the patients following a very similar pattern of metabolic phenotype characterized by increased resting metabolic rate, pronounced loss of lean body mass and fat stores and anorexia [13]. The major clinical characteristic unifying all of these conditions is the increase of circulating levels of proinflammatory cytokines, that is highly likely to „trigger“ the whole cascade of metabolic changes ultimately resulting in cachexia development. It would be interesting to know whether there is a „cytokine treshhold“ of cachexia, however, investigation of the complex network of circulating cytokines is extremely difficult and the synergism/antagonism of cytokines involved makes it even more complicated to untangle. One of the mechanisms by which the cytokines could induce anorexia is the regulation of pro-opiomelanocortin (POMC) expression [14], which is a crucial compound in the central melanocortin (MC) system. Indeed, the animal models as well as observations on humans support the idea of pivotal role of MC system in regulation of body weight, also in terms of cachexia development [15,16],

3. Physiology of MC signaling and suppression of MC pathway via AgRP

Basically, the melanocortin [MC] system is comprised of two types of neurons with opposing actions regarding appetite regulation and energy balance.

The first class of neurons present in the hypothalamus have anorexigenic effects and expresses POMC, a large precursor consecutively cleaved to smaller molecules. Intracellular posttranslational processing of the POMC propeptide, by prohormone convertase-2, in these neurons leads to the production of α-, β-, and γ-melanocyte–stimulating hormones (α-, β-, γ-MSH) [14]. These peptides signal to downstream target neurons in the lateral hypothalamus expressing the melanocortin receptors 3 and 4 (MC3R and MC4R) resulting in a decrease of food intake and increase of energy expenditure [17]. POMC is also a precursor of adrenocorticotrophic hormone (ACTH); pathological ACTH excess results in severe central obesity [18]. POMC-expressing neurons in the arcuate nucleus (ARC) are critically involved in the integration of nutritional and hormonal signals and the regulation of energy homeostasis [19]. These neurons signal to second order neurons in hypothalamic regions known to regulate feeding behaviour, neurons in multiple areas around the brain and brainstem, including the paraventricular nucleus of the hypothalamus (also involved in appetite regulation), the lateral hypothalamus, and the nucleus of the solitary tract in the brainstem [19]. Once α-MSH is released in synapses with these second-order neurons, it binds to MC3Rs and MC4Rs leading to widespread downstream effects, including a decrease in food-seeking behavior, an increase in basal metabolic rate, and a decrease in lean body mass [20-22].

The melanocortin receptor family consists of five subtypes (MC1R–MC5R) of receptors and belongs to the superfamily of G-protein-coupled receptors

(GPCRs) activating the adenylate cyclase signal transduction pathway [23]. The hypothalamic MC receptors, especially MC3R and MC4R have been recognized to regulate appetite and feeding behavior and metabolic pathways related to food intake [24]. Large number of pharmacological trials and genetic studies have demonstrated that POMC-derived MCs suppress feeding through activation of the MC4R [25,26]. Taking this into account, it seems to be highly likely that activation of the POMC neurons contributes substantially to the symptoms observed in cachexia.

The second group of neurons in the MC system has orexigenic effects and expresses neuropeptide-Y (NPY) and agouti-related protein (AgRP) [13]. The mature AgRP is a 112-amino acid, paracrine signaling protein that binds to MC3R and MC4R with high affinity [27]. Overexpression of AgRP increases feeding, slows metabolism, and leads to metabolic derangements similar to those arising from deletion of the MC4R gene [28,29]. AgRP was discovered based on its high homology with the agouti signaling protein (ASP) [27], an endogenous antagonist at MC1R involved in coat and skin pigmentation in animals. In vitro analysis revealed that ASP has high affinity for MC1R and MC4R, and moderate affinity for MC3R, while AgRP has high affinity for MC3R and MC4R but does not bind to MC1R [25]. Therefore, it is highly likely that its orexigenic effects are mediated through antagonization of α-melanocyte–stimulating hormone at the type 3 and type 4 melanocortin receptors [28,29]. These findings are well in accordance with the reports that deletion of the MC4R gene in mice results in hyperphagia, obesity and symptoms equivalent to that of adult onset diabetes [30].

An alternate mechanism that has been proposed to explain AgRP orexigenic potency is through the central nervous system opioid system involvement [31]. Animal studies have shown that administration of opioid receptor antagonists blocked AgRP-induced food intake when given simultaneously but not 24 h after AgRP injection, which indicates that the short-term effects of AgRP may be mediated through the activity of opioid receptors [32].

4. Animal models of AgRP

MC4R -/- mice display maturity onset obesity characterized by hyperfagia, increased adiposity, increased longitudinal growth, normal lean body mass, hyperinsulinaemia and increased circulating levels of leptin were observed [33]. Interestingly, the body weight of MC4R knockout mice is already higher than in the wild types before clinical onset of hyperfagia. [34]. MC receptor knockout mice generally provide a model to determine which of the (an)orexigenic signals are dependent on what type of MC receptor in modulating appetite. Transgenic mice overexpressing ubiquitously AgRP have a similar phenotype as the MC4R – / – mice, they are hyperphagic, exhibit severe obesity, and have reduced corticosterone levels [35].

Complete AgRP-deficiency, on the contrary, results in highly variant phenotypes [36]. When first reported, AgRP knockout (AgRP-/-) mice presented with normal feeding behavior without changes in body weight and cumulative food intake [37]. In a subsequent paper, however, it was noted that 2 – to 3-month-old AgRP null mice did exhibit subtle changes in response to feeding challenges (fasting and MCR agonists) but – of more significance and magnitude – exhibit reduced body weight and adiposity after 6 months of age that correlated with increased metabolic rate, body temperature, and locomotor activity [38].

However, data obtained from MC4R -/- mice are not always consistent with the previous pharmacological reports of effects of MCR4 antagonists and MC4R inverse agonists (with similar effects as AgRP agonists) [39]. When the MC4R -/- mice had to press a lever to obtain their meal, they were not hyperphagic and were more likely to lose body weight than the control animals [39] suggesting that normal MC4R functionality is not required for optimum feading patterns, but rather affects the qualitative food choice. The results of Vaughan et al suggest that, while decreased MC4R signaling may be consistent with increased food intake, the expression of that behavioral phenotype is highly dependent on the environment. The author concludes that the conditions under which MC4R knock-out mice are hyperphagic thus remain to be addressed [40].

Nevertheless, there are several reports describing the role of the MC system in preference for certain foods. First, it was reported that AgRP enhances the intake of specifically high-fat diets in rats [32]. In addition, obese mice with ectopic overexpression of Agouti (which mimics the action of AgRP) have enhanced preference for fat meals thus further supporting the hypothesis of strong MC system involvement in native qualitative composition of individually preferred diet [40].

5. Currently available small-molecule inhibitors of MC pathway

It has recently been demonstrated that blockade of the central MC system can prevent cachexia development in models of uremia, heart failure, and cancer [41-44]. As the anorexigenic pathway of α-MSH is believed to play the pivotal role in physiology and pathophysiology

of food intake regulation, it was suggested that the blockade of MC4R as its main physiological target could result in anti-cachexia effects [45]. This idea was further suppported by the observation that AgRP, the endogenous inverse agonists of MC4R, expressed significant effects on both major aspects of cachexia, as it was promoting food intake and reducing energy expenditure in animal experiments [45].

Cancer patients express multiple metabolic maladaptive responses resulting in inappropriate high energy expenditure despite low caloric intake and therefore the cancer-related cachexia cannot be treated simply as a lack of appetite. As the α-MSH, the endogenous agonistic ligand at the MC4R was found to have a dual action, i.e. to reduce food intake [46] and also to increase energy expenditure [47], blockade of MC4R pathway could be a promising approach in therapy of cancer-related cachexia as it would influence both of these aspects.

In a study by Nicholson et al [44], effectiveness of MC4R blockade in prevention of cancer cachexia was investigated in rodent models using the MC4R blocker, ML00253764. In membranes of human embryonic kidney 293 cells expressing human MC4-R, ML00253764 was capable of displacement of [Nle4, D-Phe7]-α-melanocyte stimulating hormone binding with an IC50 of 0.32 μM, whereas at concentrations above 1 μM, ML00253764 reduced cAMP accumulation, which could be indicative of inverse agonist activity. When ML00253764 was administered twice daily (15 mg/kg s.c.) for 13 days to C57BL6 mice bearing s.c. Lewis lung carcinoma tumors, ML00253764 stimulated light-phase food intake relative to vehicle-treated controls ($p < 0.05$), however, no effect on 24-h food intake was observed here [44].

The desired features of the possible therapeutic molecules with MC4R antagonist properties are high affinity and selectivity for the MC4R antagonism of MC4-mediated responses in functional assays, good central nervous system penetration and desirable pharmacokinetic properties for systemic oral administration [48]. The extended search to find selective, potent and orally active antagonists of MC4R resulted in discovery of two molecules: SNT207707 and SNT207858 [45]. The first molecule, SNT207707 binds to the MC4R with affinity of 8 nM and expresses more than 200-fold selectivity vs. MC3R and MC5R. The other molecule, SNT207858 is a 22 nM MC4R antagonist with a 170-fold selectivity vs. MC3R and a 40-fold selectivity versus MC5R [49,50]. In mice subcutaneously implanted with C26 adenocarcinoma cells, repeated oral administration of each of the two compounds almost completely prevented tumor induced weight loss, and diminished loss of lean body mass and fat mass thus confirming significant anti-cachexia effects of both compounds [45], whereas a single subcutaneous injection of 20 mg/kg of either SNT207707 or SNT207858 distinctly increased food intake of the mice ($p < 0.001$) and the amount of food taken during the four hours observation period was roughly 3-fold the amount taken by the vehicle treated controls. The results reported from this study are well in accordance with previous studies describing robust orexigenic effects and showing that endogenous peptidic or small molecule MC4R antagonists enhance food intake in healthy animals [44,50]. Besides the experiments using SNT2007707 and SNT207858 in C26 adenocarcinoma mice performed by Weyermann [45], a number of other compounds with MC4R antagonist effects were investigated elsewhere – Chen et al [52] reported on a new compound from piperazine family, the name of which is yet to be attributed, Vos et al [53] investigated effects of ML00253764 in C26 adenocarcinoma mice, Nicholson et al [44] investigated ML00253764 in Lewis Lung carcinoma mice. Cheung et al [54] investigated effects of a MC4R antagonist called NBI-12i in rodent models of cachexia with 5/6 nephrectomy where 3 mg/kg of NBI-12i or saline was given to subtotally nephrectomized or sham-operated mice intraperitoneally, twice per day, for a period of 14 days. NBI-12i–treated uremic mice gained lean body mass, fat mass, and had a lower basal metabolic rate compared to vehicle-treated and diet-supplemented uremic mice, which lost both lean body mass and fat mass and had an increase in basal metabolic rate, moreover, NBI-12i also normalized the expression of uncoupling protein, which is normally upregulated in uremic mice which may have also beneficial effects in uremia-related cachexia.

SNT207707 and SNT207858 are, however, most likely to achieve wider clinical acceptance, as they have high oral bioavailability and likely to be preferred to the subcutaneous preparations.

The AgRP agonists represent another way to suppress development of cancer-related cachexia. It has been proposed that AgRP acts as an inverse agonists of MC4R [55-57], however, although AgRP is a competitive inhibitor of α-MSH and homologous agonists, they do not share perfectly overlapping binding sites within the individual MC receptors, as the hAgRP(109-118) decapeptide results in antagonism at the MC3R while retaining MC1R agonist activity and MC4R antagonist activity [58]. In another study, AgRP did not have the opposite effect as MC3/4R agonists; also, the combined application of an inverse agonist together with the agonist is supposed to cancel the effect of the agonist, but in the experiments by Fu et al [59] AgRP and MC3/4

receptor agonists both inhibited excitatory hypothalamic ventromedial nucleus neurons, the two agents instead showing an additive effect. The results obtained by Fu et al do not argue against the prevailing view that AgRP can act to antagonize the MC3/4R in other systems, but rather that the actions on excitatory cells of the ventromedial nucleus did not fit with a mechanistic model of antagonism of the MC3/4R on the cell bodies or on presynaptic terminals innervating the glutamate neurons of ventromedial hypothalamus. The authors suggest that the mechanisms of AgRP actions on these excitatory cells appear to be independent of the actions of MCs on MC receptors.

To summarize, it is highly likely that the two key aspects of cachexia (low caloric intake and inappropriate high energy expenditure) can possibly be treated or at least improved via the MC-4R, which might have important implications for supportive care in cancer patients, in all disease types and stages.

6. Clinical trials using MC4R antagonists or AgRP agonists

The prophylaxis-treatment of cachexia as a serious and highly debilitating condition is not widely recognized as one of the major goals of the individual treatment of a cancer patient, although its progression is tightly associated with poorer prognosis of cancer patients. Cancer – related cachexia is sometimes treated/improved by corticosteroids that are capable of increasing the appetite and reversing weight loss. But this generally shows no evidence of reversing muscle loss, which is well in accordance with their known physiological action [60].

At the moment, there are no other clinically accepted drugs as well as no FDA-approved drugs designed exclusively to treat cancer cachexia.

At ASCO Meeting in 2010, there were three novel phase II clinical presentations of the treatment of cachexia, however, none of them were based on MC4R antagonists or AgRP agonists: i) ALD518 is a humanized anti-IL-6 antibody that proved safe and tolerated in phase II [61], ii) GTx-024 is a Selective Adrenergic Receptor Modulator (SARM), the use of which included significant increase in lean body mass in various types of malignancies [62], iii) VT-122 is a simultaneous treatment with propranolol and etodolac with significant, but rather delayed effects on gain of lean body mass [63]. In the study by Zhou et al [64], it was demonstrated that pharmacological blockade of ActRIIB pathway (abolishing the activation of the ubiquitin-proteasome system and the induction of atrophy-specific ubiquitin ligases in muscles) not only prevented further muscle wasting but also completely reversed prior loss of skeletal muscle and cancer-induced cardiac atrophy, suggesting that ActRIIB pathway might also provide interesting new molecules potentially utilizable in cachexia treatment.

To date, there have been no published trials employing MC4R antagonists or AgRP agonists in humans, and such trials are not listed in the National Institutes of Health clinical trials registry [65]. The extrapolation of findings obtained on rodent models on humans is always somewhat difficult, e.g. estimation of possible side effects of MC4R antagonists on systemic circulation that expresses different characteristics in humans compared to rodents. Despite the recent advances in understanding the crucial role of MC4R system in cancer-related cachexia, many questions regarding safety and long-term efficacy persist and are unlikely to be answered on the grounds of animal models only. Once the safety for human use will be assessed careful clinical investigation of long-term effects of MC4R antagonists and AgRP agonists will be required to further establish their clinical utility in the context of comprehensive cancer treatment in various types of cancer.

7. Conclusion

Cancer-related cachexia represents a complex physiological condition characterized by inappropriate high energy expenditure despite low caloric intake, so it cannot be easily treated as a lack of appetite. We conclude that peripheral administration of MC4-R antagonists or AgRP agonists represents an attractive, novel therapeutic approach for the treatment of cancer-related cachexia. Although most of the widely discussed effects of MC4R blockade refer to the enhacement of appetite, blockade of MC pathway can also substantially modulate energy expenditure, which may have potentially beneficial effects in therapy of cancer-related cachexia. However, more research will be necessary to the detailed mechanisms of therapeutical action as well as of adverse effects before the MC-pathway based cachexia treatment will be available.

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