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REVIEW ARTICLE

Inhibition of tumour cell growth by carnosine: some possible mechanisms

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Abstract The naturally occurring dipeptide carnosine (β-alanyl-L-histidine) has been shown to inhibit, selectively, growth of transformed cells mediated, at least in part, by depleting glycolytic ATP levels. The mechanism(s) responsible has/have yet to be determined. Here, we discuss a number of probable and/or possible processes which could, theoretically, suppress glycolytic activity which would decrease ATP supply and generation of metabolic intermediates required for continued cell reproduction. Possibilities include effects on (i) glycolytic enzymes, (ii) metabolic regulatory activities, (iii) redox biology, (iv) protein glycation, (v) glyoxalase activity, (vi) apoptosis, (vii) gene expression and (viii) metastasis. It is possible, by acting at various sites that this pluripotent dipeptide may be an example of an endogenous "smart drug".

Keywords Carnosine · Cancer · Tumour · Glycolysis · Glycation · Regulation · Signalling · Redox biology · NAD · Metastasis

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Introduction

Despite the fact that the naturally occurring dipeptide L-carnosine (β-alanyl-L-histidine) was characterized approximately 100 years ago, and while a variety of possible physiological roles and therapeutic uses have been ascribed to it (Quinn et al. 1992; Hipkiss 2009a), carnosine is still regarded as enigmatic (Bauer 2005; Hipkiss 2009b). Possible functions of the dipeptide include buffer, anti-oxidant, anti-glycator, aldehyde/carbonyl scavenger, metal ion chelator, immuno-stimulant, wound healing agent and neurotransmitter. In 1986, it was reported that carnosine can inhibit growth of tumour cells (Nagai and Suda 1986). About 10 years later, the ability of carnosine to exert inhibitory, but selective, effects towards the growth of cultured transformed cells was demonstrated by Holliday and McFarland (Holliday and McFarland 1996); these workers had previously shown that carnosine possessed anti-ageing activity because they found that it suppressed development of the senescent phenotype in cultured human fibroblasts, and provoked cellular rejuvenation when added to senescent cells (McFarland and Holliday 1994, 1999). In 2008, carnosine was shown to inhibit growth of cultured glioblastoma cells (Renner et al. 2008), most probably via effects on glycolysis (Renner et al. 2010a; Asperger et al. 2011; Iovine et al. 2011). Other studies indicated that carnosine can suppress tumour growth in animals (Renner et al. 2010b; Horii et al. 2012). The mechanism(s) by which carnosine exerts its anti-tumour effects is/are at present unknown (Gaunitz and Hipkiss 2012). The objective of this paper is to outline some of the various possible mechanisms by which carnosine could mediate its inhibitory effects towards transformed cells, whilst exerting little or no deleterious effects towards non-transformed post-mitotic cells with which carnosine is normally associated (muscle and nervous tissue).

Metabolic differences between normal cells and tumour cells

The best studied metabolic phenotype of cancer is aerobic glycolysis—also known as the Warburg effect which is characterized by increased metabolism of glucose to lactate in the presence of sufficient oxygen [for review see Bensinger and Christofk (2012) and Koppenol et al. (2011)]. The molecular mechanisms driving the Warburg effect are highly heterogeneous (Bayley and Devilee 2012). Otto Warburg hypothesized that the effect may be caused by an intrinsic mitochondrial perturbation (Warburg 1956). Although, it is now appreciated that mitochondrial defects in tumours are rare (Frezza and Gottlieb 2009) and ATP production by glycolysis may even not be necessary in every type of tumour (Rodríguez-Enríquez et al. 2012), it is still without debate that glycolysis is enhanced in tumours (Israel and Schwartz 2011; Vander Heiden 2009, 2011; Vander Heiden et al. 2010). A number of recent papers have highlighted that most anabolic processes required for accelerated tumour growth rate are accomplished by increased glycolysis (Vander Heiden et al. 2010; Hamanaka and Chandel 2012). Since aerobic glycolysis is, in part, regulated by HIF1 and mTOR, there is also a link to hypoxia (Wilson and Hay 2011). Because of the great importance of glycolysis for tumour cell growth, glucose deprivation or the inhibition of glycolytic activity is considered as a strategy towards new therapies (Jiang et al. 2011; Csibi and Blenis 2011; Porporato et al. 2011). It has been suggested that decreasing the activity of the glycolytic enzyme phosphofructokinase (Mor et al. 2011) would decrease glycolytic supply of ATP and metabolic precursors and thereby suppress tumour growth. In addition, it has been proposed that the institution of low glucose and high ketogenic diets should be employed in brain cancer patients (Seyfried et al. 2011, 2012).

It is interesting to note that (i) suppression of glycolytic activity and stimulation of mitochondrial function are frequently associated with ageing delay and lifespan extension, and (ii) carnosine's inhibition towards cultured glioblastoma cells is accompanied (caused?) by decreased glycolytically generated ATP levels (Renner et al. 2010a). However, the precise nature and mechanism by which such inhibition is mediated have yet to be revealed.

Carnosine and glycolysis

Glycolysis is the predominant pathway by which the major cellular carbon source is converted into the carbon skeletons of the building blocks (e.g. amino acids and nucleotides) required for cell growth. As noted above, there is evidence suggesting that in tumour cells carnosine somehow inhibits ATP synthesis during glycolysis. Furthermore, inhibition of glycolysis would also suppress formation of necessary metabolic building blocks required for growth and proliferation.

Studies in yeast and in frog retinal development support this proposal. Treatment of the yeast *Saccharomyces cerevisiae* with carnosine was inhibitory to growth when cultured fermentatively on glucose, whereas the dipeptide stimulated aerobic growth when the cells were cultured on glycerol as sole carbon source (Cartwright et al. 2012). Carnosine has been detected in frog retina (photoreceptors and bipolar cells) (Panzanelli et al. 1997) and a study on retinal development carried out 20 years ago showed that carnosine is detectable in post-mitotic retinal neurones, but only from stage 39/40 onwards (Pognetto et al. 1992). It has recently been shown that during retinal cell development, energy metabolism switches from glycolysis to oxidative phosphorylation, being complete at developmental stage 41 (Agathocleous et al. 2012). These observations, in organisms as diverse as yeast and frog (retina), seem to suggest that carnosine is deleterious towards glycolytic metabolism but advantageous when metabolism is essentially aerobic, which mirror the dipeptide's contrasting effects towards tumour cells (glycolytic) and differentiated fibroblasts (aerobic) and are consistent for the idea that carnosine could play a role in management of energy metabolism. However, the relative levels of carnosine in glycolytic and aerobic muscles may seemingly argue counter to these proposals, but it is suggested that the higher carnosine concentrations in glycolytic muscle may serve a homeostatic function in this tissue by buffering hydrogen ions and suppressing the glycating potential of triose phosphates and their highly toxic degradation product methylglyoxal (MG).

There have been few assessments of carnosine levels in tissues during growth, but limited studies revealed that in humans, carnosine levels in gastrocnemius and soleus muscle tissue increase in pre-pubescent children (Grinio and Stvolinsky 2011), reaching a highest value between the age of 15 and 20 and declines with further age (Baguet et al. 2012). Two other studies also indicated that tissue carnosine levels decline in old age in humans as well as in rodents (Tallon et al. 2007; Stuerenberg and Kunze 1999). These and other observations seem to imply that carnosine may be generally antiproliferative. Consequently, to explain carnosine's effects towards tumour cells, the possibility that it prevents glucose uptake into the cell and/or inhibits one or more specific step(s) in glycolysis should be considered.

Carnosine and regulation of glycolysis

Control of glycolysis is mediated by complex signalling mechanisms involving nutrient sensing and pathway

regulation (Salminen and Kaarniranta 2012). In the following sections, we will briefly analyse how regulatory mechanisms may be affected by carnosine.

AMPK, CREB, CRTC and calcineurin

Two major regulators of energy homeostasis and glucose metabolism in mammals are the AMP-activated protein kinase (AMPK) and members of the CREB family (cyclic AMP response element binding transcription factor family). Whereas the activity of AMPK is regulated by changing levels of AMP and ADP, arising from ATP depletion, CREBs are activated by cAMP. Among the important targets of AMPK are the CREB-regulated transcriptional co-activators [CRTCs; originally designated as transcriptional co-activator for the transcription factor CREB (TORCs), not to be confused with mTORCs, the targets of rapamycin complexes]. CRTCs in association with CREB activate cAMP-response element (CRE)-mediated gene transcription including genes important for energy metabolism and cancer. At this point, it is important to understand that this activation is different from the gene activation mediated by cAMP via Proteinkinase A which leads to the phosphorylation of CREB at Ser133 and its association with CBP/p300 (Conkright et al. 2003). Upon activation of AMPK, CRTCs become phosphorylated which sequesters them to the cytosol terminating their effects on transcription. Inactive CRTCs in turn can be re-activated by dephosphorylation mediated by calcineurin. Just recently, it has been demonstrated that lifespan extension in *Caenorhabditis elegans* is dependent on inactivation of CRTC-1 which is the sole CRTC in this worm (Mair et al. 2011).

In our opinion, a possible connection between carnosine and CRTC should be considered because carnosine, in the form of its zinc complex (polaprezinc), has recently been shown to inhibit calcineurin activity in rats (Zhang et al. 2011). Consequently, it is possible that by inhibiting calcineurin, carnosine, when complexed with zinc, could conceivably modulate energy metabolism, perhaps via changes in the activity of CRTC.

Carnosine, eIF4E and mTOR

The mammalian target of rapamycin (mTOR) is a conserved serine/threonine kinase and an important downstream effector of the PI3K/AKT pathway. Deregulation of different components of the mTOR pathway has been reported in many types of cancer [see Pópulo et al. (2012) for a recent review]. mTOR exists in two biochemically and functionally distinct multi-component complexes known as mTORC1 and mTORC2 [Cybulski and Hall (2009); Alessi et al. (2009)]. mTORC1 is sensitive to rapamycin and phosphorylates a number of proteins involved in protein translation including eukaryotic initiation factor 4E binding proteins (eIF4EBPs) and ribosomal protein S6 kinases 1 and 2 (S6K1/2). mTORC1 activation is also associated with cell growth, increased glycolytic activity and suppressed mitochondrial function, whilst mTORC1 inhibition frequently results in mitochondrial stimulation, suppressed glycolysis and a delay in onset of age-related dysfunctions. It is not known whether carnosine can influence mTOR activity, directly or indirectly. However, there is evidence that carnosine might affect intracellular signalling and mTOR-related activity. Carnosine was shown to inhibit phosphorylation of MAP-kinase, ERK1/2 and Akt (Son et al. 2008) which resulted in changes in eIF4E translation initiation factor activity, analogous to that mediated by treatment with rapamycin (Yellen et al. 2011) and resveratrol (Villa-Cuesta et al. 2011), both well-known anti-tumour and anti-ageing agents. Carnosine's ability to inhibit ERK1/2 activation has recently been demonstrated in cultures neurones (Rybakova et al. 2012). Consequently, changes in MAP-kinase activity or other pathway components induced by carnosine may constitute another mechanism by which carnosine could influence energy metabolism, which in turn would inhibit glycolysis in tumour cells and thereby restrict their growth. In addition, there is also a link between cAMP and mTOR since this second messenger was shown to inhibit both mTOR complexes by promoting complex dissociation (Xie et al. 2011).

Carnosine and cell cycle arrest

Carnosine can promote cell cycle arrest (Iovine et al. 2011; Jia et al. 2009; Rybakova and Boldyrev 2012), as well as affect lifespan and tumour cell growth (discussed above). It is known that accumulation of the cell cycle regulatory protein, p27, is associated with growth inhibition in tumour cells (Eto 2013), whose expression may be controlled by the STAT3 signalling pathway (Guo et al. 2013). It has recently been reported that carnosine can activate the STAT3 signalling pathway (Wang et al. 2013), and furthermore there is one report (in Chinese) showing that carnosine provokes an increase in p27 levels (when added to HL-60 cells) and inhibits cell proliferation (Zhang et al. 2005). Thus, it is possible that carnosine's ability to influence expression of at least one cell cycle regulatory protein (p27) may help to explain its suppressive effects on tumour cell proliferation. However, the exact mechanisms by which these effects could be mediated remain to be explored.

Direct effects of carnosine

Does carnosine affect glucose uptake into cells?

A literature survey reveals almost nothing on whether carnosine directly affects glucose entry into cells, although evidence is accumulating that the dipeptide can suppress many of the deleterious effects of hyperglycemia.

Does carnosine affect the activity of glycolytic enzymes?

If carnosine exerts an inhibitory effect towards any particular glycolytic enzyme, it would be expected that the relative amount of that enzyme's substrate would accumulate and its product lessened in amount, compared to the untreated state. To our knowledge, however, no such classical pathway analysis has been performed. Indeed, such an analysis could be readily achieved using erythrocytes. Furthermore, should it be found that carnosine does inhibit the activity of a glycolytic enzyme, this could provide an explanation for the presence of carnosinase activity in blood.

There is no published evidence showing that carnosine has any direct effects on (i) the conversion of glucose to glucose-6-phosphate via hexokinase activity, (ii) conversion of glucose-6-phosphate to fructose-6-phosphate via phosphoglucose-isomerase, or (iii) the conversion of fructose-6-phosphate to fructose-1,6-diphosphate via phosphofructokinase.

There is one report showing that carnosine can affect glucose metabolism in stressed mice (Tsoi et al. 2011), but it is thought that this occurs indirectly via hormonally mediated effects on gene expression. In restraint-stressed mice, it was found that oral administration of carnosine reversed the stress-induced down-regulation of the messenger-RNAs in the liver for the glucose transporter GLUT2, glucose-6-phosphatase and glucokinase, most probably via the dipeptide's effects on plasma corticosterone. It is thought that these effects are mediated via carnosine's anti-stress effects by way of the histaminergic neuron system (Tsoi et al. 2011).

Fructose-1,6-bisphosphatase Fructose-1,6-bisphosphatase is an important enzyme in gluconeogenesis and has been reported to be down-regulated in hepatocellular carcinoma (Liu et al. 2011). Upregulation of hepatic fructose-1,6-bisphosphatase is, however, associated with raised cAMP levels and fasting (Sakai et al. 2012; Wang et al. 2012), both of which are linked to lifespan extension and ageing delay induced by resveratrol and dietary restriction. There is a somewhat old observation showing that carnosine stimulates fructose-1,6-bisphosphatase activity obtained from rabbit muscle (Ikeda et al. 1980). If carnosine similarly stimulates fructose-1,6-bisphosphatase in tumour cells, then this would promote an ATP-consuming futile cycle between fructose-1,6-bisphosphate and fructose-6-phosphate, the consequences of which would be decreased overall glycolytic flux and lowered ATP levels, as observed (Renner et al. 2010a; Iovine et al. 2011). Thus, the establishment of such a futile cycle that consumes ATP, induced by carnosine-mediated stimulation of fructose-1,6-bisphosphatase activity, could conceivably provide an explanation of the dipeptide's inhibitory effects towards tumour cell growth.

Pyruvate kinase The enzyme pyruvate kinase (PK) is thought to exert important control functions towards glycolysis, especially in tumour cells (Mazurek 2011). PK catalyses the generation of ATP and pyruvate from phosphoenolpyruvate. PK can exist in two alternative isoforms, PKM1 and PKM2. All cancer cells seem to possess the PKM2 form exclusively, whereas differentiated cells express PKM1 (Vander Heiden et al. 2010). These isoforms differ in their response to fructose bisphosphate (FBP); PKM2 shows a marked (at least fourfold) increase in specific activity in the presence of FBP, whereas the PKM1 was not activated by FBP (Vander Heiden et al. 2010). Consequently, the observation that carnosine can stimulate fructose bisphosphatase activity (Ikeda et al. 1980) might lead to a decline in PKM2 activity and the observed decrease in cellular ATP (Renner et al. 2010a).

Phosphoglycerate mutase phosphorylation In rapidly proliferating cells. it has been shown that the metabolism of phosphoenol pyruvate can result in the phosphorylation of a specific histidine residue in the glycolytic enzyme phosphoglycerate mutase (PGM), instead of producing ATP from ADP (Vander Heiden et al. 2010; Engel et al. 2004). It has also been shown that a peptide fragment from PGM can inhibit phosphorylation of the intact enzyme. Furthermore, when a synthetic cell-penetrating form of the peptide fragment (PGMtide) was added to a tumour cell line, growth was arrested and glycolytic flux inhibited (Engel et al. 2004), although non-tumour cells were not affected. Consequently, in regard to carnosine's anti-proliferative effects towards tumour cells, could carnosine (β-alanyl-L-histidine) also inhibit PGM phosphorylation? This idea is reinforced by the finding that pyruvate suppressed the inhibitory effects of PGMtide towards tumour cells (Engel et al. 2004); pyruvate was also found to suppress the anti-proliferative effects of carnosine towards tumour cells (Holliday and McFarland 1996). Thus, it seems reasonable to suggest that a study should be undertaken to determine whether carnosine inhibits histidine phosphorylation of PGM in tumour cells.

Does carnosine react with glycolytic intermediates?

Carnosine has been shown (Hipkiss 2009a, b) to react directly, but non-enzymatically, with glucose and fructose, and presumably with their phosphorylated derivatives glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-diphosphate, but there have been no reports of any of these carnosine adducts being present in tissues. The enzyme aldolase converts fructose-1,6-diphosphate to dihydroxyacetone-phosphate and glyceraldehyde-3-phosphate, both of which are very much more reactive towards amino groups generally and to carnosine in particular. Should carnosine react with intracellular dihydroxyacetone-phosphate or glyceraldehyde-3-phosphate, then this might compromise glycolysis in tumour cells. Furthermore, both these triose phosphates can spontaneously decompose into MG, a highly reactive bicarbonyl which is thought to be responsible for much dysfunction in type-2 diabetes and ageing generally (Rabbani and Thornalley 2008; Maher and Schubert 2009; Cantero et al. 2007; Turk 2010).

Carnosine and redox biology

Carnosine has been reported to exert beneficial effects on redox status at the whole animal level (Kalaz et al. 2012). Thus, it is likely that the dipeptide influences the prooxidant-antioxidant relationship in tumour cells. The fact that many tumour cells do not actively produce ATP by oxidative phosphorylation means that these cells may regenerate NAD^+ from NADH via operation of glycerol-3-phosphate dehydrogenase which reduces dihydroxyacetone-phosphate (DHAP) to glycerol-3-phosphate (Grivell et al. 1995), the glycerol-3-phosphate is then dephosphorylated and the glycerol may then be secreted via an aquaglyceroporin (Verkman 2005; Verkman et al. 2008). Most tumour cells show a marked upregulation of a number of aquaglyceroporins (Shen et al. 2010; Xu et al. 2011; Kusayama et al. 2011; Yang et al. 2011) which allows glycolysis to continue via operation of glyceraldehyde-3-phosphate dehydrogenase (which required NAD^+). Carnosine, being an aldehyde/ketone scavenger, readily reacts with dihydroxyacetone (DHA) and can protect proteins against DHA-mediated glycation (Hipkiss et al. 1995a, b). Therefore, by reacting intracellularly with DHAP, carnosine may suppress NAD^+ regeneration from NADH in tumour cells. Such a possibility could inhibit glycolysis and decrease ATP synthesis (as observed) as well as generation of necessary metabolic intermediates for protein and nucleic acid synthesis.

It may be relevant to note here that carnosine's ability to suppress growth of tumour cells was inhibited by addition of mM amounts of either pyruvate, oxaloacetate or alpha-ketoglutarate, whilst the reduced forms of these metabolites, lactate, malate and isocitrate, as well as succinate and fumarate, had no effect on the dipeptide's ability to inhibit growth (Holliday and McFarland 1996). Consequently, the observation that compounds possessing reducible carbonyl groups, i.e. pyruvate, oxaloaceate or alpha-keto-glutarate, which also circumvents the growth-inhibitory action of carnosine, points to the possibility that carnosine's inhibitory effects on the tumour cells may be mediated, at least in part, via effects on NAD^+ regeneration from NADH. This could occur as a consequence of direct reaction of carnosine with DHAP. Additionally, or alternatively, carnosine could inhibit one or more of the enzymes responsible for the conversion of DHAP to glycerol-3-phosphate and then to glycerol, and the latter's subsequent extracellular disposal using an aquaglyceroporin. However, to our knowledge, there are no reports of whether carnosine has any effects on these processes.

Carnosine and PARP activity

Poly(ADP-ribose) polymerases (PARPs) are activated during the inflammatory response and help recognize and repair DNA strand breaks. There is evidence showing that carnosine downregulates PARP-1 expression in human glioblastoma cells (Scalia et al. 2013) as well as in primary cultures of astrocytes and oligodendrocytes (Spina-Purrello et al. 2010) following oxidative stress. Curiously, it was reported that carnosine increased survival of the stressed glioblastoma cells (Scalia et al. 2013). It is thought, however, that the effect of carnosine could be a consequence of antioxidant activity (Scalia et al. 2013) although other mechanisms cannot be excluded.

Carnosine, methylglyoxal and apoptosis

Tumour cells are primarily glycolytic in their ATP provision. Such increased glycolysis also raises the potential for generation of MG. As noted above, MG is generally regarded as a major source of macromolecular glycation, and is especially associated with hyperglycemia and the secondary complications of diabetes (Rabbani and Thornalley 2008; Maher and Schubert 2009; Cantero et al. 2007; Turk 2010). However, there are reports that MG can suppress apoptosis in cultured tumour cells. For example, it has recently been shown (Oya-Ito et al. 2011) that MG glycates the stress protein Hsp27 at a number of arginine residues, and, as a result, the glycated Hsp27 was found to be even more effective in preventing apoptosis than the native unglycated protein. Significantly, it has been shown that a number of carbonyl scavengers can inhibit the anti-apoptotic effects of MG, simply by decreasing the degree

of Hsp27 glycation and thereby lowering the protein's anti-apoptotic activity (Wondrak et al. 2006). Carnosine can scavenge MG and protect proteins against MG-induced modification, at least in a model system (Hipkiss and Chana 1998). Thus, by directly reacting with MG, carnosine could decrease Hsp27 glycation thereby suppressing the enhanced anti-apoptotic effects of glycated Hsp27. Alternatively the dipeptide could react with (i.e. "carnosinylate") the glycated Hsp27; "carnosinylation" of MG-modified protein has been demonstrated at the test-tube level (Brownson and Hipkiss 2000), although it is uncertain whether it also occurs in vivo. A third possibility is that carnosine could provoke deglycation of the MG-modified protein: a role for carnosine in deglycation/transglycation has been proposed (Szwergold 2005). It should be noted that with regard to the action of carnosine on glioblastoma cells, we have not observed evidence of apoptotic activity (caspase activation) (unpublished results).

There is evidence showing that certain AGEs can exert anticancer activity; mice with high levels of plasma AGEs develop smaller tumours than mice with normal AGE levels, and in human subjects high serum AGE levels seem to delay tumour reoccurrence (Bartling et al. 2011). An anticancer formulation based on MG has been developed (Talukdar et al. 2008). This at least raises the possibility that carnosine's reactivity towards MG could also create a similar product which exhibits anticancer properties.

Carnosine, deoxyribose and tumour cells

Tumour cells possess upregulated thymidine phosphorylase whose activity generates thymine and 2-deoxy-D-ribose, the latter inhibiting apoptosis and stimulating p70/S6k signalling required for angiogenesis (Bijnsdorp et al. 2011; Nakajima et al. 2009). Carnosine can react with deoxyribose (Hipkiss et al. 1995a, b). Hence, it is possible that carnosine could react intracellularly with thymidine phosphorylase-generated deoxyribose and thereby suppress anti-apoptotic activity, inhibit tumour growth and prevent angiogenesis.

Carnosine and glyoxalase activity

Due to their high glycolytic activity, tumour cells possess increased levels of glyoxalase enzyme, presumably required for destruction of excess MG (Thornalley and Rabbani 2011; Bair et al. 2010). Consequently, inhibition of glyoxalase activity has been proposed as a possible target for controlling tumour cell growth, and some inhibitory molecules have been proposed, including curcumin (Yuan et al. 2011; Santel et al. 2008). Assuming that carnosine reacts with MG intracellularly, it is possible that the resultant carnosine-methylglyoxal adduct could compete with the glutathione-methylglyoxal adduct (i.e. D-lactoyl-glutathione) normally formed by the action of glyoxalase-1 and thereby inhibit either or both glyoxalase-1 and glyoxalase-2 activity. It should be relatively easy to test the validity of this speculation.

Carnosine and uncoupling proteins

A number of recent papers have suggested that the uncoupling protein UCP2 plays an important role in cancer cell physiology and therefore may constitute a target for therapeutic intervention (Ayyasamy et al. 2011; Baffy et al. 2011; Robbins and Zhao 2011). Whilst there are no reports relating carnosine to UCP2, it is interesting to note that UCP2 is very unstable having a half-life of 30 min (Rousset et al. 2007). Given that it has also been noted that renal cell carcinomas seem to possess a number of defects in the ubiquitin–proteasome proteolytic apparatus (Guo et al. 2012) which may contribute to the overexpression of HIF-1α, and that carnosine is reported to stimulate proteolysis of HIF-1α (Bharadwaj et al. 2002), it is at least conceivable that carnosine might stimulate proteolysis of UCP2. There is a further co-incidental (?) overlap between uncoupling proteins and carnosine; both have been shown to influence anxiety-like behaviour in rodents (Gimsa et al. 2011; Tomonaga et al. 2008). This again raises the possibility that either carnosine might influence UCP activity, or both carnosine and UCP act via a common pathway. If carnosine does lower UCP2 levels, then it is possible that this would result in increased MG formation, due to a decreased ability to regenerate NAD^+ from NADH which would limit GADH activity and promote triose phosphate accumulation.

Carnosine and the hypoxic response

Most tumour cells are primarily hypoxic. Consequently, tumour cells must adapt by synthesizing a number of proteins necessary for hypoxia. The process (the hypoxic response) is controlled by a hypoxia-inducible factor (HIF-1α protein). In oxygenated cells, this protein is rapidly catabolised by the ubiquitin-proteasomal system. Due to its central role in controlling cancer cell metabolism, inactivation, inhibition or repression of HIF-1α has been proposed as a potential method of controlling tumour cell growth (See Xia et al. 2012 for recent review). There is one report showing that carnosine can suppress the hypoxic response possibly by stimulating proteolysis of HIF-1α (Bharadwaj et al. 2002). Although there have been no

follow-up papers confirming or refuting these claims, there is some evidence showing that carnosine can stimulate proteolysis in cultured human fibroblasts (Hipkiss, 2009a). However, it has recently been reported that mutations in genes encoding components of the ubiquitin-mediated proteolytic apparatus occur with an elevated frequency in renal carcinoma cells which may contribute to the raised levels of HIF-1α and HIF-2α (Guo et al. 2012). So the presence of carnosine might to some degree counter the proteolytic dysfunction resulting in tumour cells.

A SUMO connection?

PK is also subject to modification by SUMO (Spoden et al. 2009) which may affect the subcellular distribution of the protein. In addition, hypoxia can upregulate SUMOylation up to tenfold, which has been suggested to increase resistance to therapeutic agents, possibly by interacting with HIF-1α (Shao et al. 2004). Conversely, it has been shown that inhibition of protein SUMOylation renders MCF-7 cells more susceptible to DNA-damaging agents. Interestingly, it was also shown that protein SUMOylation was required for at least a fourfold increase in intracellular carnosine following radiation treatment (Cano et al. 2010). Although it is unclear as to the precise nature of the relationship between carnosine synthesis, SUMOylation and PK activity, these observations are nevertheless intriguing.

Carnosine and gene expression

There have been few publications describing the effects of carnosine on gene expression. Ikeda et al. showed that carnosine stimulated expression of vimentin in cultured rat fibroblasts (Ikeda et al. 1999). Although vimentin expression is a recognized tumour marker, it is not known whether carnosine upregulates vimentin expression in tumour cells and whether changes in vimentin expression affect tumour cell viability.

More recently, however, a proteomic analysis of carnosine-treated glioblastoma cells (Asperger et al. 2011) has yielded useful information on the effects of carnosine on gene expression; it was found that out of over 700 proteins detected, the expression of 31 of them was affected by the dipeptide. Of these 31 proteins, seven have been identified, including an approximately 50 % decrease in expression of a protein called von Hippel–Lindau binding protein-1 (VBP-1). VBP-1 participates in control of the hypoxic response mediated by the stabilization of the hypoxic response factor-1α (HIF-1α) under low oxygen tensions. Thus, it is speculated that decreased availability of VBP-1 will decrease binding of the von Hippel–Lindau protein to HIF-1α which in turn will permit the proteolytic degradation of HIF-1α thereby decreasing the instigation of the hypoxic response which tumour cells require. It has previously been shown that carnosine promotes HIF-1α degradation in cardiomyocytes (Bharadwaj et al. 2002), an observation consistent with the above suggestion.

Carnosine and metastasis

There is one publication showing that carnosine inhibits metastasis of hepatoma cells. Chuang and Hu (2008) found that carnosine, when added to SK-Hep-1 cells, resulted in increased expression of an antimetastatic gene, nm23-H1. One effect of the nm23-H1 gene product was the inhibition of the matrix metalloproteinase MMP-9, an activity normally associated with tumour cell invasion. It has also been shown that nm23-H1 is a nucleotide diphosphate kinase which can form a complex with a number of glycolytic enzymes (including glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate mutase) which can decrease glycolytic flux in tumour cells and inhibit their growth (Engel et al. 2004). It was also found that the presence of pyruvate suppressed the inhibitory effects of the nm23-H1-enzyme complex. It is interesting to note that Holliday and McFarland reported that pyruvate suppressed the inhibitory effects of carnosine towards tumour cell growth (Holliday and McFarland 1996), thus inviting a parallelism between the anti-tumour actions of carnosine and nm23-H1. Whether nm23-H1 is also directly involved in PGM histidine phosphorylation has been disputed however (Vander Heiden et al. 2010).

Serum carnosinase activity, is it a problem?

The presence of serum carnosinase activity is frequently thought to be a barrier to any potential therapeutic efficacy of carnosine in humans, especially if administered orally. Consequently, the perception that serum carnosinase is a potential impediment to the dipeptide's survival in vivo for any useful length of time, may explain the paucity of clinical trials involving dietary carnosine supplementation. Forms of the dipeptide resistant to carnosinase attack have therefore been developed; these include *N*-acetyl-carnosine, D-carnosine and cyclodextrin-conjugates. It is not known, however, if any of these exert inhibitory effects on growth of cultured tumour cells. Never-the-less, there are three reports of randomized, double-blind trials of dietary supplementation with carnosine in humans, which, however, involve changes in behaviour rather than any molecular parameter. The effects of carnosine supplementation on (i) children with autistic spectrum disorders

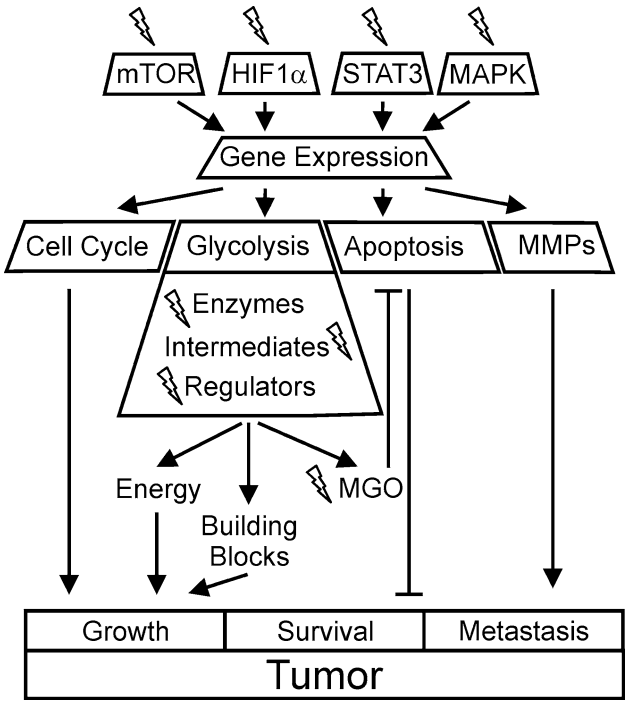

Fig. 1 Overview over possible mechanisms by which carnosine may influence tumour cell growth, survival and metastasis. The *flash* symbols indicate the possible targets of carnosine as discussed in the manuscript

(Chez et al. 2002), (ii) schizophrenic adults (Chengappa et al. 2012) and (iii) veterans suffering from Gulf War Illness (Baraniuk et al. 2013), have been studied: all three reported beneficial effects in behaviour and/or cognitive function. Whilst these findings are encouraging in terms of producing effects induced by dietary carnosine (presumably acting on the brain), there is no direct evidence of any mechanistic route. However, it would seem that, given these encouraging effects on some aspect brain activity, exploratory trials using carnosine-supplemented diets could be explored with respect to intractable brain tumours such as glioblastoma multiforme.

Conclusions

Carnosine has previously been termed pluripotent and enigmatic. The present discussion certainly seems to reconfirm this view. There is evidence that carnosine may suppress glycolysis via a number of possible mechanisms: activating a gluconeogenic enzyme, inhibiting mTOR by effects on protein synthesis initiation, raising cAMP levels and suppressing NAD^+ regeneration from NADH. In addition, carnosine may decrease hsc27 glycation, promote HIF-1α proteolysis, stimulate p27 and nm23-H1 expression and inhibit glyoxalase activity, all of which would compromise tumour cell viability. Figure 1 summarizes these various possibilities. At present, we are unable to state with any certainty which, if any, of these proposals adequately explain the dipeptide's effects towards controlling tumour cell growth. It is possible that the naturally occurring compounds such as carnosine and fisetin (Chiruta et al. 2012) have multiple biological activities, and that such pluripotency should be recognized as advantageous in drug development (Maher and Schubert 2009). Indeed, it has recently been suggested that "it is time to move on from the concept—one drug, one target—toward smart drugs that can simultaneously modulate multiple targets" (Mai 2012). Is carnosine an example of a smart drug? The apparent efficacy of dietary supplementation towards certain aspects of brain dysfunction suggests that the presence of serum carnosinase activity is not necessarily a barrier to carnosine's therapeutic potential.

Conflict of interest The authors declare that they have no conflict of interest.

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