Potential Protective Protein Components of Cow’s Milk against Certain Tumor Entities

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Abstract: Milk and dairy products, especially from cow’s milk, play a major role in the daily human diet. It is therefore hardly surprising that the subject of milk is being extensively researched and that many effects of individual milk components have been characterized as a result. With the wealth of results available today, the influence of milk on the development of various types of cancer and, in particular, its often protective effects have been shown both in vitro and in vivo and in the evaluation of large-scale cohort and case-control studies. Various caseins, diverse whey proteins such as α-lactalbumin (α-LA), bovine α-lactalbumin made lethal to tumor cells (BAMLET), β-lactoglobulin (β-LG), or bovine serum albumin (BSA), and numerous milk fat components, such as conjugated linoleic acid (CLA), milk fat globule membrane (MFGM), or butyrate, as well as calcium and other protein components such as lactoferrin (Lf), lactoferricin (Lfcin), and casomorphines, show antitumor or cytotoxic effects on cells from different tumor entities. With regard to a balanced and health-promoting diet, milk consumption plays a major role in a global context. This work provides an overview of what is known about the antitumoral properties of proteins derived from cow’s milk and their modes of action.

Keywords: cow’s milk; antitumoral effects; health-promoting diet; cancer; antitumor peptides

1. Introduction

Almost all mammalian individuals including humans usually take up the preform of the mother’s milk, the so-called colostrum, which is formed by the female mammary glands after birth. A short time later, ordinary milk is produced and, in the first period, exclusively nourishes the newborn with its composition adapting to the developmental stage and growth requirements. At this time, milk serves as the sole source of nutrition containing all the ingredients required for development such as proteins, enzymes, carbohydrates, vitamins, trace elements, and, to ensure a functional immune response, antibodies as well as defense-promoting enzymes.

Humans began to build up a regulated dairy industry about 10,000 years ago with the domestication of goats and sheep, especially in Western Asia, and later, 8500 years ago, in Southern Europe with the keeping of the aurochs. In particular, the evolutionary advantage for Northern Europeans was the so-called lactase persistence, a strong positive selection of the lactase allele with no longer occurring lactose intolerance, as a result of the habituation to dairy farming [1].

In recent years, global cow’s milk production has expanded from a total of 441.97 million tons in 2010 to 524.41 million tons in 2019—an increase of 18.65% over this period [2,3]. In 2019, the largest producing countries were in the EU (155.2 million tons),
with the main share of 33.1 million tons in Germany [4], the USA (99.06 million tons), and India (92 million tons), with particularly strong growth rates in India (+45.3% since 2010 with 50.3 million tons) [2,3]. Per capita milk consumption including milk equivalents of dairy products without butter has increased on a global average from 78.24 kg in 2000 to 111.6 kg in 2019, which means an increase of 29.9% for this timespan [5,6]. In addition, all national and international dietary guidelines recommend a regular intake of milk and dairy products (e.g., yogurt and cheese) as part of a health-promoting eating pattern [7]. The dairy group supplies many nutrients, including calcium, phosphorus, vitamin D, vitamin A, riboflavin, vitamin B12, protein and essential amino acids, potassium, zinc, choline, magnesium, and selenium [8].

In 2020, the World Health Organization (WHO) reported a global cancer burden of 18.1 million cases (thereof 9.6 million deaths) for the year 2018 and a probable increase to 29.4 million cases in 2040. Therefore, besides its treatment, the effective primary prevention of carcinoma development is of paramount importance [9]. The highest incidence for males was found for lung, prostate, and colorectum cancers, with a proportion of 14.5%, 13.5%, and 10.9%, respectively. For females, breast, colorectum, and lung were most frequently affected (24.2%, 9.5%, and 8.4%, respectively).

For these reasons, besides the pure development of new anti-cancer drugs, emphasis has always been placed on the study of naturally occurring compounds, which often also show interesting cancer preventive effects. Some nutritional compounds succeeded, e.g., in limiting the growth of cancer cells and, at the same time, positively influenced the body’s own immune system [10–13]. In this context, daily nutrition plays an important role, which additionally increases the special interest in milk components and their effects. In addition, evidence from case-control and prospective cohort studies demonstrate an inverse association between the regular intake of milk and dairy products and the development of various types of cancer, especially of colorectal cancer [14–17]. However, causality cannot be inferred from these statistical associations. Observed inverse relations between intake of dairy products and colorectal cancer development have been largely attributed to their high calcium content. Other nutrients or bioactive constituents in dairy products, such as Lf, vitamin D or the short-chain fatty acid butyrate may also impart some protective functions against cancer, but these require much better elucidation.

2. Composition of Cow’s Milk

Bovine milk constituents are mainly water (86–88%), milk fat (3–6%), protein (3–4%), lactose (5%), and minerals (0.7%) as shown in Figure 1. The percentage of total solids is 11–14% [18].

![Figure 1. Approximate composition of cow’s milk. Proteins are divided into insoluble casein proteins and soluble whey proteins [18].](image-url)
families according to the homology of their primary amino acid sequences, namely \(\alpha_s1\)- (\(\alpha_s1\)-CN, 12–15 g/L in skim milk), \(\alpha_s2\)- (\(\alpha_s2\)-CN, 3–4 g/L), \(\beta\)- (\(\beta\)-CN, 9–11 g/L), and \(\kappa\)-caseins (\(\kappa\)-CN, 2–4 g/L), each consisting of several different variants due to genetic heterogeneity (Figure 2) [19].

Figure 2. Overview of the insoluble casein proteins and their share in the total casein fraction. The green box indicates some important processed peptide variants after casein digestion. Some of them are \(\alpha\)-amides, methyl esters or have free \(\alpha\)-carboxyl groups [20–22].

The casein proteins occur as macromolecular aggregates known as casein micelles, with a size from 30 to 300 nm. The whey protein fraction includes \(\alpha\)-LA (0.6–1.7 g/L), \(\beta\)-LG (2–4 g/L), BSA (0.4 g/L), immunoglobulins (predominantly IgG1, 0.3–0.6 g/L), bLF (0.02–0.1 g/L), lactoperoxidase, and other proteins to a minor proportion (Figure 3) [19].
3. Milk Proteins and Processed Peptides with Chemopreventive Properties

Milk proteins are components, which have certain physiological functionalities, that can often be diversified according to their potential to release bioactive peptides after enzymatic digestion in vitro and in vivo. Large numbers of potentially effective peptides are “encrypted” in the complete proteins until they are activated by enzymes in the gastrointestinal environment. Protein members of the casein and whey fraction with known antitumor activities are introduced in the following sections, which are additionally summarized in tabular form (Table 1).

| Proteins | Tumor Species | Effects and Potential Mechanism | Ref. |
|----------|---------------|--------------------------------|------|
| Casein proteins | α-, β-, κ-caseins | Breast cancer (human): MCF10A H Ras (G12V), MDA-MB-231 | Decreased cell migration | [24] |
| | | Mammary tumor (murine): Met-1 | Decreased cell migration, tumor growth, Metastasis, activation of STAT1 signaling, apoptosis induction (shown for α-CN) | |
| | Lactaptin (κ-casein fragment) | Breast carcinoma (human): MCF-7 | Apoptosis induction | [25] |
| | PGPIPN (β-casein fragment) | Ovarian cancer (human): SKOV3 | BCL2 targeting | [26] |
| Proteins | Tumor Species | Effects and Potential Mechanism | Ref. |
|----------|---------------|-------------------------------|------|
| INKIKI (β-casein fragment) | Melanoma (murine): B16F10 | Apoptosis induction | [27] |
| CMs | Prostatic cancer (human): LNCaP, PC-3, DU145 | Interaction with opioid receptors | [28] |
| | Breast cancer (human): T47D | G0/G1 blocking | [29] |
| | Promyeloic leukemia (human): HL-60 | Apoptosis induction | [30] |
| CPPs | Intestinal tumor (human): HT-29 | Interaction with voltage-operated L-type calcium channels, apoptosis | [31] |
| | Colon carcinoma (murine): Co26Lu | Inhibitory effects on lung metastatic colony formation in Balb/c mice due to Tand NK cell activation | [32] |
| | Melanoma (murine): B16-BL6 | Inhibition of lung metastasis in C57BL/6 mice (only apo-Lf) | [33] |
| | Lymphoma (murine): L5178Y-ML25 | Inhibition of liver and spleen metastasis in C57BL/6 mice (only apo-Lf) | [33] |
| | Colon carcinoma (murine) | Reduced induction of aberrant crypt foci (ACF) by azoxymethane administration in male F344 rats | [34] |
| Lf | Pepsin hydrolysate f(17–38) | Apoptosis induction by JNK/SAPK MAP kinase activation | [35] |
| | Pepsin hydrolysate (mixture) | Oral squamous cell carcinoma (human): SAS | [36] |
| | Basal diet with 0.2% bLf | Hamster buccal pouch (HBP) carcinoma | [37] |
| | Native and iron saturated bLf | Glioblastoma (human): GL-15 | [38] |
| Liposomal bLf | Colorectal cancer (rat): DMF-DSS induced colorectal | Suppression of inflammation and tumor cell proliferation | [39] |
| | α-LA in complex with oleic acid (BAMLET) | Fibrosarcoma (murine): Meth A | Reduction of tumor growth in CB6 mice | [40] |
| | Melanoma (murine): B16F10 | Cytotoxic activity, lysis by pore formation (SEM) | Cytotoxic activity | Cytotoxic activity |
| | Colon carcinoma (murine): C26 | Accumulation in endolysosomal compartment, lysosomal membrane permeabilization inducing nonapoptotic lysosomal cell death | [41] |
### Table 1. Cont.

| Proteins | Tumor Species | Effects and Potential Mechanism | Ref. |
|----------|---------------|--------------------------------|------|
| β-LG | Lymphocytic leukemia (murine): L1210 | | |
| | Lung adenocarcinoma (human): A549 | Apoptosis induction, upregulation of Bax and caspase-3, decreased level of Bcl-2, reduced chemotactic motility, tumor inhibition in BALB/c mice after oral administration | [42] |
| | Intestinal tumor (human): HT-29 | | |
| | Hepatoblastoma (human): HepG2 | | |
| | Breast carcinoma (human): MDA231-LM2 | | |

BAMLET: bovine α-lactalbumin made lethal to tumor cells; CM: casomorphin; CN: casein; CPP: casein phosphopeptide; DMBA, 7,12-dimethylbenz[a]anthracene; DMF-DSS: 1,2-dimethylhydrazine/dextran sulphate sodium, EMT: epithelial-to-mesenchymal transition, IL-6: interleukin-6, JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinase; Lf: lactoferrin; Lfcin: lactoferricin; SEM, scanning electron microscope; STAT1/3: signal transducer and activator of transcription 1/3; α-LA: α-lactalbumin; β-LG: β-lactoglobulin.

#### 3.1. Casein Proteins and Processed Peptides

##### 3.1.1. Caseins and Casomorphines

The different casein fractions occur in bovine milk in following proportions: αs1 (39–46% of total caseins), αs2 (8–11%), β (25–35%), κ (8–15%), and γ (3%), which is a natural degradation product of β-casein (Figure 2) [21,22,43,44]. Depending on cattle breed, all casein families are additionally present in multiple genetic variants with different amino acid substitutions [19], which give rise to a multitude of biologically active protein fragments after hydrolysis during gastrointestinal digestion or food processing like fermentation [20,45,46]. In milk, for example, β-caseins occur in 13 different genetic variants, of which the most common are A1 and A2 [47]. These two differ from each other only in a single amino acid substitution at position 67 (histidine in A1 β-casein to proline in A2 β-casein) caused by a single nucleotide exchange, respectively.

In vitro experiments with bovine α-, β-, and κ-casein proteins showed a reduction of migration ability of murine mammary tumor cells Met-1 and two human breast cancer cell lines (MCF10A H Ras (G12V) and MDA-MB-231) with α-casein being most potent [24]. Additionally, recombinant α-casein expression in Met-1 cells led to reduced tumor mass and lung metastasis in athymic nude mice by induced STAT1 signaling. Furthermore, different proteolytic fragments of κ- and β-casein showed antitumor effects against adenocarcinoma (MCF-7), ovarian cancer (SKOV3), or murine melanoma (B16F10) [25–27].

Bovine casomorphins are a group of opioid-like peptides derived from limited proteolysis of α- and β-caseins. The first casomorphin identified after an enzymatic casein digest was the heptapeptide βb-casomorphin-7 (BCM-7; Tyr-Pro-Phe-Pro-Gly-Pro-Ile) and the corresponding βb-casomorphins-4, -5, and -6 (peptides with 4, 5, and 6 amino acid residues) [48]. Opioid peptides derived from bovine α-casein- and κ-casein-digestion are named αb-casein exorphins (1–7 and 2–7) and casoxins A, B, and C, respectively [49].

In the context of antitumoral activity, different casomorphin peptides were shown to have antiproliferative effects on human prostate cancer cell lines LNCaP, PC-3, and DU 145 through partial interaction with opioid receptors [28]. Other results showed the in vitro blocking of breast cancer T47D cells in the G0/G1 phase through antiproliferative action of five investigated casomorphins (αs1-caseins f(90–95) and f(90–96), BCM-7 f(60–66), BCM-5 f(60–64), and morphiceptin (amide of BCM-4)) [29]. Interaction between the casomorphins and the tumor cells takes place via δ- and κ-opioid receptors with exception of morphiceptin interacting with type II somatostatin receptor. In this case, the CMs showed different receptor affinities. In another work by Noni et al., BCM-7 is described as a weak opioid receptor agonist [50]. Furthermore, BCM-7 and the phosphopeptide β-casein f(1–25)4P have been shown to induce apoptosis of HL-60 cells (promyeloic leukemia) [30].

Nevertheless, it should be mentioned that the proliferation of prostate cancer cell lines LNCaP and PC3 was described to be increased after treatment with α-casein and total casein (0.1 or 1 mg/mL, respectively) from bovine milk. Interestingly, growth rates
of lung cancer cells (A459), stomach cancer cells (SNU484), breast cancer cells (MCF7), immortalized human embryonic kidney cells (HEK293), and immortalized normal prostate cells (RWPE1) were not affected [51].

3.1.2. Casein Phosphopeptides

Casein phosphopeptides (CPPs) are released by gastrointestinal trypsin digestion from \(\alpha_{s1}, \alpha_{s2}, \) or \(\beta\)-caseins (which differ in their extent of phosphorylation) and function as carriers for different minerals (like magnesium, iron, and trace elements such as zinc, barium, chrome, nickel, cobalt, and selenium) by forming complexes (especially with calcium) modulating their bioavailability [52]. Therefore, the precipitation of calcium phosphate in the intestines is inhibited and the amount of soluble calcium for absorption is increased [53]. Cytomodulatory effects of CPPs are, e.g., stimulation of interleukin-6 cytokine release in intestinal epithelial cells and stimulation of immunoglobulin A production [54,55]. CPPs are also shown to interact with voltage-operated L-type calcium channels, thereby modulating proliferation and apoptosis depending on intestinal tumor HT-29 cell differentiation [31]. CPP-induced calcium uptake is also elevated in human intestinal Caco2 cells, but only upon cell differentiation [56].

3.2. Whey Proteins and Processed Peptides

3.2.1. Lactoferrin

Human and bovine Lf (hLf, bLf) are single chain multifunctional glycoproteins with non-haem iron-binding properties and belong to the family of transferrin proteins, which are capable of binding and transferring \(\text{Fe}^{3+}\) ions [57]. hLf and bLf consist of 689 and 691 amino acid residues, respectively, with a molecular mass of about 80 kDa [58]. Lf possesses two binding sites for one ferric ion (\(\text{Fe}^{3+}\)) and one bicarbonate anion each [59]. It is produced by mucosal epithelial cells and also stored in the secondary granules of polymorphonuclear leukocytes (PMNLs) of miscellaneous mammalian species [60]. It was first purified in 1939 as unknown “red fraction” from Cow’s milk [61]. Later in 1960, it was demonstrated to be the main iron binding protein in human milk [62–64]. It exists as hLf and bLf with a sequence homology of nearly 70% [58]. bLf is also the main iron-binding protein in cow’s milk [65] and is present in other biological secretory fluids like saliva, tears, nasal, and bronchial secretions, as well as in semen, urine, vaginal, and gastrointestinal fluids [66,67]. Lf is fundamentally involved in the regulation of cellular and systemic iron homeostasis [68–70]. Caseins make up the largest share of the protein components of milk with Lf having also a large proportion, ranging from a concentration of about 7 g/L in human colostrum to 1–3 g/L in mature milk [71,72], whereas Cow’s milk contains lesser Lf, ranging from 0.05 to 0.5 g/L from early-to-mid lactation [19,73,74]. Through its antiviral, bactericidal, and antifungal properties, Lf being part of the innate immune response plays a functional role among others for the health state of the mammary gland [75,76]. Various other protective functions for Lf are described, e.g., antianemic, antioxidant, anti-inflammatory, immunomodulatory, or anticancer properties [33,40,77–83]. Gram-negative bacteria with negatively charged lipopolysaccharides anchored in the outer membrane, can be complexed with the highly positively charged lactoferrin, which then forms holes, through which milk lysozyme can enter and destroy the proteoglycan matrix [84]. Intact Lf is taken up in significant amounts by a specific Lf receptor (LfR) occurring in the apical membrane of the small intestine by an endocytotic process [85,86]. It must be taken into account that in infants and newborns, the intragastric pH is higher and the expression and secretion levels of the digestive enzymes are lower than in the adult individual with a matured digestive system. Therefore, non-absorbed unhydrolyzed hLf can be detected in the faecal extracts of breast-fed babies [87]. Moreover, different tissues like liver, bone, or brain and cell types like lymphocytes or fibroblasts express further LfRs [76]. Eventually, Lf is acting as a transcription factor inside the nucleus affecting immunomodulation by cytokine expression (e.g., interleukin-1β and transforming growth factor-β) or epithelial growth and differentiation [88]. Due to the high sequence
homology of bLf, it was postulated to be broadly bioequivalent to hLf and was recently used in several in vivo and also clinical studies mainly to investigate its anti-inflammatory potential in oral or even aerosolized form [89–91]. In a mouse model, orally administered bLf showed inhibitory effects on lung metastatic colony formation after subcutaneous injection of colon carcinoma Co26Lu cells [32]. Furthermore, the tumor growth and lung metastasis of murine melanoma B16 and lymphoma L5178Y-ML25 cells could be inhibited by bLf in another mouse model, interestingly mainly apo-Lf [33,92]. Moreover, Cutone and colleagues showed that native and iron-saturated bLf differently inhibited cell migration in a human glioblastoma model via the reversal of epithelial-to-mesenchymal transition-like process and inhibition of the IL-6/STAT3 pathway, with the holo-form being the more effective form [38]. These observations highlight the importance of iron saturation state of Lf when conducting in vitro and in vivo studies [68]. Additionally, Lf has been suggested to affect tumor cell growth through natural killer (NK) and lymphokine-activated killer (LAK) cell activation [92,93]. After bLf treatment, an inhibition of colon carcinoma development caused by azoxymethane administration has been observed in rats [34]. In another mouse model, elevated levels of IL-18 produced by intestinal mucosa were detectable after bLf treatment, further caspase-1 activity and interferon-γ (IFN-γ) levels were increased [94]. The proinflammatory cytokine IL-18 is an important factor in mucosal immunity by generating CD4+ and CD8+ T cells and the activation of T and NK cells followed by IFN-γ production [95,96]. Caspase-1 is necessary to process the mature active form of IL-18 and is itself activated by enzymatic cleavage of its procaspase-1 precursor [97]. Other experiments with oral bLf showed the additionally increased expression of IFN-α and IFN-β in mouse Peyer’s patches (PP) and mesenteric lymph nodes (MLN) stimulating intestine-associated immune functions [98]. hLf, with its high similarity to bLf (about 70% sequence homology and similar 3-dimensional structure) [99], was able to inhibit the cell proliferation of MDA-MB-231 breast carcinoma cells at G1 to S transition of the cell cycle by decreasing the activity of cyclin-dependent kinases [100]. Experiments with hLf demonstrated the further downregulation of 3-phosphoinositide-dependent protein kinase 1 (PDK1) transcription via mitogen-activated protein kinase/c-Jun pathway following deactivation of AKT signaling leading to inhibition of nasopharyngeal carcinoma (NPC) tumorigenesis [101]. Moreover, pepsin hydrolysates of bLf showed apoptosis induction in human myeloid leukemia cells (HL-60) and human oral squamous cell carcinoma cells SAS, demonstrating the activity of bLf inherent peptides [35,36]. In an in vivo experimental setting with hamsters, the incidence of 7,12 dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis could be decreased by feeding a basal diet containing 0.2% bLf. In this case, levels of phase I enzymes were decreased, lipid peroxidation was modulated, and antioxidant and phase II enzyme activities increased [37]. In a rat hepatocellular cancer model, Lf showed chemopreventive effects by regulating protein kinase B pathway [102] and liposomal bLf was described to suppress inflammation and tumor cell proliferation in a 1,2-dimethylhydrazine/dextran sulphate sodium (DMH-DSS)-induced colorectal cancer rat model [39]. Notably, no adverse effects were described after applying 1.5–9 g recombinant hLf in a phase I trial to treat refractory solid tumors [103].

3.2.2. Lactoferricin

In adulthood, bLf ingested orally by drinking Cow’s milk is largely enzymatically cleaved into smaller peptides by gastric pepsin in the stomach with a low pH of 1.0–2.5 [104], followed by losing some of its abilities like binding iron or a comparably lower anti-viral activity. Nonetheless, degradation products such as bovine lactoferricin (LfcinB, 25 amino acid residues 17–41 of bLf) or lactoferrampin (20 amino acid residues 265–284 of bLf) are amphipathic peptides with the more hydrophobic residues lying on one side, more positively charged residues on the other [105], and possess antimicrobial activity. Lactoferrampin differs by showing more antimicrobial properties, whereas Lfcin additionally exhibits anti-inflammatory and anticancer activities [105]. The antitumor effects of LfcinB are described against a variety of tumor entities, like murine leukemia, fibrosarcoma (Meth A),
melanoma (B16F10), or colon carcinoma (C26) [40]. Lfcin disrupts the cell membrane and triggers apoptosis through an oxidant-dependent pathway. The amphipathic structure is necessary to enter the cell by targeting the peptide to the relatively negative charged cell surface because of more abundantly present phosphatidylserine molecules [106] followed by the insertion of the hydrophobic residues into the membrane disrupting cell integrity by pore formation [40].

3.2.3. α-Lactalbumin and Bovine α-Lactalbumin Made Lethal to Tumor Cells (BAMLET)

α-LA is the second most common whey protein following β-LG in human and bovine milk and has a molecular weight of 14.2 kDa (123 amino acid residues). It is produced in the lactating mammary gland of almost all mammals and is required for the synthesis of lactose [107]. Human and bovine α-LA share 71% sequence homology and similar Ca\(^{2+}\)-binding sites [108].

Back in 1995, the human equivalent of BAMLET called human α-lactalbumin made lethal to tumor cells (HAMLET) was discovered to kill a variety of transformed, embryonic, and lymphoid cells sparing mature epithelial cells [109]. Later, it was shown that the fatty acid oleic acid (C18:1) is necessary as cofactor to form an effective complex with α-LA to induce apoptosis [110]. Hereby, the folding and stability of α-LA is affected by pH and Ca\(^{2+}\) ions and play a crucial role in forming BAMLET. α-LA must be present in the partially unfolded apo-state (devoid of Ca\(^{2+}\)) before complex formation [110]. The observed cytotoxicity against tumor cells by inducing cell death works via lysosomal membrane permeabilization and was demonstrated by experiments from Rammer and colleagues. According to their results, this is due to the specific accumulation of BAMLET in the endolysosomal compartment of tumor cells, followed by the release of cathepsins and other lysosomal hydrolases into the cytosol, which activates proapoptotic Bax protein [41]. Thereby, cytotoxicity against a variety of human (HeLa, J82, RT4, PC-3, U118, MCF-7, and U2-OS) and murine (L1210) tumor cells was demonstrated, likewise low cytotoxicity against healthy murine embryonic fibroblasts (NIH-3T3). However, the results of various groups that also show the cytotoxicity of BAMLET to primary cells should not be ignored. Brinkmann et al. showed that peripheral blood mononuclear cells (LC\(_{50}\) of 20 µg/mL BAMLET) were quite sensitive against BAMLET treatment compared with primary endothelial cells (LC\(_{50}\) of 1.37 mg/mL), in addition to be cytotoxic against different carcinoma cell lines (e.g., HL-60 (human promyeloic leukemia), Skov-3 (human ovarian adenocarcinoma), and B16F0 (murine melanoma)) [111]. In these experiments, α-LA alone was shown to be not toxic, otherwise oleic acid treatment of Jurkat (acute T cell leukemia) and THP1 cells (acute monocytic leukemia) alone led to cell death resembling apoptosis and necrosis.

3.2.4. β-Lactoglobulin

β-LG is the most abundant non-casein protein in bovine milk, with a share of about 50% of the whey protein fraction, and is not present in human milk. Its molecular weight is 18.3 kDa (162 amino acid residues) and it exists as a dimer of two main genetic variants A and B, which differ by two-point mutations under physiological pH and ambient temperature [23]. Below pH 3, β-LG dissociates into monomers [112]. The protein is thought to act as whey carrier protein for a variety of hydrophobic molecules, like retinoids (e.g., vitamin A), lipids, and polyphenols protecting them against oxidative damage or increasing their solubility [113]. For ligand binding, pH is important influencing the access to and release from the hydrophobic binding pocket [114]. β-LG is quite resistant to peptic and chymotryptic digestion, because of its structural and conformational properties where the cleavage sites are not easily accessible [115]. Therefore, β-LG could eventually survive the gastrointestinal passage unharmed and increase intestinal absorption of protected bound molecules. Anti-tumor effects have been seen in vitro within a study with different human tumor models (lung tumor cell line A549, intestinal epithelial tumor cell line HT-29, hepatocellular cell line HepG2, and breast cancer cell line MDA231-LM2) and further in vivo in a xenograft model with BALB/c nude mice with orally administered
protein [42]. Thereby, tumor growth and development were reduced through induction of mitochondria-dependent apoptosis by upregulation of Bax and Caspase-3 levels and decreasing Bcl-2 level. Furthermore, β-LG was used as nanocarrier for hydrophobic acid labile drugs for oral administration like irinotecan, a potent agent in colorectal cancer treatment, which showed more cytotoxic effectiveness against human gastric carcinoma AGS cells and colon carcinoma HT-29 cells than the free drug [116]. Interestingly, β-LG was shown to form cytotoxic complexes by binding sodium oleate analogously to the HAMLET/BAMLET complexes of α-LA with oleic acid [117]. Results with human monocytic cells U937 (myeloid leukemia) suggested an apoptotic pathway, whereas healthy cells were less affected, as demonstrated with rat adrenal pheochromocytoma cells PC12, which exhibit phenotypic features of mature normal cells in the differentiated state.

3.3. Milk Fat Globule Membrane

Milk fat globules have a unique colloidal assembly structure to pack and release triacylglycerols and other bioactive molecules in the form of droplets in milk. The functional milk fat-encapsulating membrane called milk fat globule membrane (MFGM) consists of a phospholipid trilayer. The lipid core is covered with a monolayer surface coat of proteins and polar lipids after being released from the endoplasmic reticulum into the cytosol. These microlipid droplets (MLDs) fuse together intracellularly to form larger cytoplasmatic lipid droplets (CLDs, average 3–4 µm in diameter) and are probably gradually coated with plasma bilayer membrane during release into the alveolar lumen of the mammary gland [118]. This ensures the dispensation of the droplets in milk serum without aggregation with others. Proteome analysis of bovine MFGM revealed a composition of 69–73% lipid and 22–24% protein [119]. Major proteins are, e.g., periodic acid Schiff 6/7 (PAS6/7, the bovine homologue of human lactadherin), butyrophilin (BTN), or mucin-1 (MUC1) and play a role in the fight against bacteria and viruses [120]. Among the multiple proteins identified, almost half of them exert membrane/protein trafficking or cell signaling functions [121]. In vitro experiments with MFGM isolates in a colon cancer cell model with human HT-29 cells revealed varying antiproliferative capacities of differently processed samples. Both thermal denaturation and hydrolysis using trypsin or phospholipase A2, affecting the protein and phospholipid fraction, respectively, led to a reduction of MFGM-mediated antiproliferative activity, suggesting the responsibility of not only the components, but also the structure of MFGM [122]. One possible mechanism among others could be the degradation of xanthine oxidoreductase (XO), a superoxide- (O2−) and H2O2-producing molybdenum flavoprotein with low specificity, which is also one of the main components of the MFGM [123].

4. Conclusions

The steadily increasing knowledge about bioactive peptides that originate from different food sources is a research topic that can provide lead structures or identify compounds that are suitable for the prevention or even treatment of numerous diseases. In recent years, milk has gained growing interest due to its potential health-promoting effects, which is illustrated by elevated numbers of publications highlighting anticancer activities of its compounds. In addition, there is evidence from observational studies demonstrating an inverse association between the regular intake of dairy products and colorectal cancer. Together with other well-known milk ingredients (e.g., milk lipids), the role of caseins, whey proteins, and their derivatives in the field of regulation of immunity, prevention of infection, antioxidant and anti-inflammatory effects, and anticarcinogenic action is becoming more and more evident.

Nonetheless, there are also studies that show an increase in cancer risk from milk. For prostate and breast cancer in particular, there are in vitro data as well as results from cohort studies that show the inductive effects of milk on the development of cancer [124,125]. Especially in the case of prostate cancer induction, data diverge widely between increased consumption of reduced-fat milk and whole milk [126,127]. In vitro digested Cow’s milk
was also shown to particularly stimulate growth of prostate cancer cells among other investigated cells of different tumor entities in culture [128]. Further experiments concerning the effects of whole milk on the prostate carcinogenesis in rats revealed an increased tumor incidence [129]. Some studies indicate an inductive effect of milk consumption on the risk of developing breast cancer, although in turn the data situation is less clear in other studies [130–132]. In the case of individuals with lactose intolerance characterized by low consumption of milk and other dairy products, a decreased risk of developing lung, breast, and ovarian cancer was determined [133]. In this context, an increased incidence may be possibly suspected in hormone-associated tumors.

On the other side, many of the milk ingredients discussed in this work show promising anticancer properties, and hence, these results could play an important role in the development of chemopreventive peptide-based drugs, which are not genotoxic, have high selectivity, and are well tolerated. Nevertheless, it should always be considered whether orally administered milk proteins “survive” the gastrointestinal digestion pathway as a whole or are hydrolyzed to form smaller peptides with changed properties and special degradation patterns. This depends on their three-dimensional structure with differently accessible cleavage sites for the digestive enzymes, the gastric emptying time, or intragastric pH, which is different in the fasting state (in adults pH 5.0–6.0) and takes up to 100 min to reach the pH optimum for pepsin digestion of 1.5–2.0 [134]. The digestion rate therefore also depends on the individuals age, the fasting/feeding state, the enzymesubstrate ratio, and the form of administration. Hence, liquid food has a faster gastric emptying time compared to solid products. Furthermore, milk is subjected to industrial processing steps like homogenization or heating and cooling during pasteurization and ultra-high temperature treatment for sterilization, which influences protein digestibility in advance prior to consumption. To ensure gastrointestinal passage for sensitive proteins methods like microencapsulation, the covalent attachment of polyethylene glycol (PEG), named PEGylation, or saturation of ion binding sites to reduce or prevent proteolysis can be used.

There are also differences between organic and conventionally produced milk, mainly depending on feeding patterns (e.g., year-round pasture grazing) [135]. However, the majority of studies focused on fatty acid distribution with increased levels of beneficial polyunsaturated fatty acids, such as conjugated linoleic acid in organic milk [136], with a pasture-based diet identified as a main contributor to these effects [137]. On the other hand, pasture feeding is not exclusive for organic produced milk and the differences in milk composition are diminished if this type of feeding is also used in the case of conventionally produced milk [135]. A study that compared both organic and conventional farming with year-round pasture grazing showed that other factors, such as the clover content of the pasture, can have a greater influence on milk composition than farming systems themselves [135]. Another study evaluated differences of protein content between cow’s milk from late pasture and early indoor feeding season. The authors found significant differences between organic and conventionally manufactured milk in the late pasture season regarding the beneficial proteins Lf (334.99 and 188.02 mg/L), β-LG (4.12 and 2.68 g/L), and lysozyme (15.68 and 12.56 µg/L), whereas the situation was largely different in early indoor feeding season with lower concentrations of BSA, bLf, and lysozyme in organically manufactured milk [138]. These findings highlight the complexity of the comparative effects on milk composition between organic and conventional farming, due to multiple parameters such as feed composition, seasonal effects, cow breed, or the possibility of free-range farming. In addition to identifying the possible health-promoting and anticarcinogenic properties of individual milk proteins, their quantification in milk of different species is also gaining importance. For example, goat milk contains far more Lf than cow milk, while the Lf content of human and sheep milk is also on a high level [139].

A similar trend can be seen in the evaluation of the milk protein content of different dairy products, such as yogurt, cheese, and further fermented dairy products. Among these, cheese, e.g., is characterized by a particularly high antioxidative potential, which is derived, among other things, from the protein fraction of the milk, and in particular, the
caseins, but is also co-determined by conjugated linoleic acid, coenzyme Q, and various vitamins. Moreover, fermentation by proteolytic cleavage by probiotics seems to increase the release of antioxidant peptide fragments [140]. For instance, Lfcin is hydrolyzed by lactic acid bacteria [141]. In some studies, bLf contents of liquid milk and yogurt were found to be at a comparable level and enriched in whole milk powder [142], whereas Lf content of certain yogurts was relatively low in another study [143]. This also opens up important new research areas, for example identifying yogurt cultures that metabolize the desired milk proteins only to a small extent. Similarly, the opportunity of enriching certain components in certain modified dairy products is also an interesting approach for the production of functional food.

According to the multiple possible interactions on different levels of cell biology and to determine exactly which milk ingredients have which influence on cancer development, there is still a lot of research that has to be done on this promising research area—on the molecular level in vitro as well as within studies of the whole organism in vivo.

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Abbreviations

BAMLET bovine α-lactalbumin made lethal to tumor cells
BCM βb-casomorphin
bLf bovine lactoferrin
BSA bovine serum albumin
BTN butyrophilin
CLA conjugated linoleic acid
CLD cytoplasmatic lipid droplet
CN casein
CPP caseinphosphopeptide
DMBA 7,12 dimethylbenz[a]anthracene
DMH-DSS 1,2-dimethylhydrazine/dextran sulphate sodium
FAO Food and Agriculture Organization of the United Nations
FAS Foreign Agricultural Service
HAMLET human α-lactalbumin made lethal to tumor cells
HBP hamster buccal pouch
hLf human lactoferrin
LAK lymphokine-activated killer
Lf lactoferrin
Lfcin lactoferricin
LfcinB bovine lactoferricin
LfR Lf receptor
MFGM milk fat globule membrane
MLD microlipid droplet
MLN mesenteric lymph nodes
MUC1 mucin-1
NK natural killer
PAS6/7 periodic acid Schiff 6/7
NPC nasopharyngeal carcinoma
PDK1  3-phosphoinositide-dependent protein kinase 1
PEG  polyethylene glycol
PMNL  polymorphonuclear leukocyte
PP  Peyer’s patches
USDA  United States Department of Agriculture
WHO  World Health Organization
XO  xanthine oxidoreductase
α-LA  α-lactalbumin
β-LG  β-lactoglobulin

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