Salivary Amino Acid Levels in Cancer

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amino acids | metabolome | saliva | cancer

1. Introduction

Amino acids, as a raw material for protein synthesis and a product of protein metabolism, enter the body or are synthesized endogenously. They play mainly physiological roles as major metabolites and regulators of metabolism among the most important compounds.

Amino acid metabolism is part of the altered processes in cancer cells [1]. Amino acids are the building blocks as well as sensors of signaling pathways that regulate basic biological processes. The main role of amino acids is to provide substrates for the biosynthesis of proteins and nucleic acids and to participate in the metabolism of carbohydrates and lipids. They are also involved in non-enzymatic antioxidant mechanisms (through the synthesis of glutathione) and epigenetic modifications (mainly with the participation of S-adenosylmethionine as a methyl group donor) [2][3][4]. Pathways of amino acid metabolism that tumor cells activate as antioxidants have been described, including the metabolism of cysteine and methionine and their association with folic acid, transsulfuration pathways, and ferroptosis [5]. Amino acids are actively studied as potential targets for anticancer therapy (asparagine, arginine, methionine and cysteine) [1][6][7]. Asparagine depletion has been successfully used for decades in the treatment of acute lymphoblastic leukemia; arginine auxotrophic tumors are excellent candidates for treatment with arginine starvation, etc.

A number of studies have shown that amino acids used as potential biomarkers vary for different types of cancer, and changes in the concentration of amino acids in body fluids and tissues are important for diagnosing cancer, as well as choosing treatment tactics [8][9][10].

One of the promising biological fluids for the determination of amino acids is saliva [11][12][13][14][15][16][17][18][19]. It contains molecules that can potentially be associated with the course of the disease and facilitate diagnosis and prognosis, including proteins, mRNA, miRNA, enzymes, hormones, antibodies, antimicrobial components, growth factors, and metabolites [20]. The determination of amino acids in saliva has been described in the norm [21][22], including intra-day and inter-day variations, as well as the age of the volunteers [23]. The existing literature data on

the content of amino acids in saliva in cancer patients are few, scattered and contradictory. There is currently no detailed justification for changes in the concentrations of individual amino acids and/or their combinations in saliva in certain types of cancer, including no understanding of how these changes are related to the content of amino acids in cancer cells, which emphasizes the relevance of research in this direction.

2. Various Types of Cancer

OSCC. Sugimoto M. et al. [24] identified the following amino acids for the diagnosis of oral cancer: alanine, leucine, isoleucine, glutamic acid, phenylalanine, and serine. Wei J. et al. [25] focused on phenylalanine and valine, which had also previously been identified as discriminating serum metabolites in OSCC compared to healthy subjects [26]. At the same time, amino acid concentrations decreased in cancer, which may be associated with increased glycolysis during cell proliferation in cancer tissues [24]. Reddy I. et al. [27] showed that levels of amino acids histidine, threonine, valine, isoleucine, methionine, phenylalanine, leucine, lysine, tyrosine, arginine, alanine, glycine, serine and aspartic acid were significantly higher in both well-differentiated OSCC cases and moderately differentiated OSCC cases than in healthy controls. Wang Q et al. [28][29][30] showed that the content of phenylalanine and leucine decreased compared to the control. Mean concentrations of phenylalanine in OSCC patients with T1–2 compared with healthy controls were 1.6 times lower ($p = 0.028$) and leucine 3.8 times lower ($p = 0.001$). As a standalone biomarker, leucine may have a better predictive power for the early stages of OSCC, and phenylalanine can be used to screen for and diagnose advanced stages of OSCC. The combination of phenylalanine and leucine improved sensitivity and specificity. Ohshima M. et al. [31] isolated valine, leucine, isoleucine, tryptophan, and alanine, while Lohavanichbutr P. et al. [32] focused on glycine and proline. The concentrations of glycine and proline in the saliva of oral cancer patients are lower than those of the control group in both sets of samples. The authors hypothesized that OSCC tumor cells take up glycine from the salivary extracellular space and actively synthesize glycine in mitochondria to form one-carbon units for subsequent nucleotide synthesis to support tumor progression. Yatsuoka W. et al. [33] found significant differences between the groups in terms of histidine and tyrosine levels, and de Sá Alves M. et al. [34] isolated methionine and leucine in a set of 25 metabolites. Thus, most studies point to the important role of leucine and isoleucine, phenylalanine, valine and alanine in the diagnosis of oral cancer. At the same time, in different studies, the concentration of amino acids varied in different ways; no definite regularity had been revealed: in half of the studies, the concentration of amino acids was up-regulated, and in the other half—down-regulated. Apparently, this was due to the characteristics of the studied sample and/or differences in sample preparation and analysis of the biomaterial. Three of the four studies for which amino acid concentrations were up-regulated used capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS), while ultra-performance liquid chromatography was used to obtain down-regulated amino acid profiles.

Breast cancer. Sugimoto M. et al. [24] proposed eight amino acids (lysine, threonine, leucine + isoleucine, glutamic acid, tyrosine, valine, and glycine) as part of a number of metabolites for diagnosing breast cancer. Cheng F. et al. [35] analyzed 17 amino acids to distinguish stage I–II breast cancer from healthy controls: Pro, Thr, and Glu ($p < 0.001$); Phe, Trp, Met, Asp, Ser, Gln and Leu ($p < 0.01$); His, Val and Lys ($p < 0.05$); and Ala and Arg ($p > 0.05$).

However, only three amino acids were included in the complex index for detecting early breast cancer: proline, threonine and histidine. Comparison of amino acid levels in stage I–II and III–IV breast cancer showed no significant differences, with the exception of valine (p = 0.027). Zhong L. et al. [36] identified two amino acids: phenylalanine and histidine. An obvious decrease in the level of acetylphenylalanine indicates a violation of the metabolism of phenylalanine in individuals with breast cancer. A similar abnormality in phenylalanine metabolism was found in oral squamous cell carcinoma [30]. Murata T. et al. [37] identified four amino acids leucine, glutamine, isoleucine, and serine. The authors also determined salivary metabolite concentration in four cancer subtypes: Luminal A-like, luminal B-like, HER2-positive, and triple-negative. However, differences between molecular biological subtypes of breast cancer are characterized by other metabolites, not amino acids. Thus, in the four studies analyzed, the authors identified different amino acids; in three studies, leucine and isoleucine are common, but there is no justification for the choice of these particular amino acids from the point of view of the biochemistry of the ongoing processes, which has yet to be done.

Gastric cancer. For gastric cancer, all studies used spectroscopy methods: Raman and ultraviolet, as well as sensors. Chen et al. did not evaluate the content of individual amino acids (Gly, Gln, His, Ala, Glu, Pro, Tyr); data processing was carried out using principal component analysis; sensitivity and specificity were noted above 80 and 87%, respectively [38]. In [39], the DAA index was proposed, which ranged from 2 to 10 for cancer patients; while in the healthy control group, DAA scores remained low (from 0 to 1). The authors quantified the probe fluorescence shift by defining the percent fluorescence quenching (PFQ) as $(I_0 - I)/I_0 * 100\%$, where I_0—the fluorescence intensity of the blank control, I—the fluorescence intensity of the test samples. The DAA index was evaluated as a number of PFQ/5%. The test results showed that the concentration of DAA in healthy people and stomach cancer patients was 0~25.3 μM, in particular (D-Ala)/0~11.3 μM (D-Pro) and 50.6~253.2 μM (D-Ala)/22.5~112.6 μM (D-Pro), respectively. These results showed that gastric cancer patients and healthy people can be distinguished using the silver DNA nanocluster. Moreover, test values were in good agreement with the range of physiologically significant concentrations (6.6 ± 1.2 μM (D-Ala)/12.8 ± 5.5 μM (D-Pro) for healthy individuals and 205.8 ± 79.5 μM (D-Ala))/80.3 ± 34.2 μM (D-Pro) for patients with gastric cancer). Li Z. et al. also used sensors; however, unlike [40], carbon dots confined in N-doped carbon (CDs@NC) were used, but the amino acids determined were the same D-Ala and D-Pro. The sensitivity and specificity of these methods have not been evaluated, and no detailed patient information is available. Of note, all three studies highlight alanine and proline as important amino acids for diagnosing gastric cancer.

Lung cancer. Jiang X. et al. [41] identified 23 metabolites, which allow diagnosing early lung cancer with high accuracy; this list includes four amino acids (serine, proline, valine, arginine). A decrease in amino acid content and activation of downstream metabolites of amino acid metabolism, including ketoleucine, N-acetylhistidine, imidazolepropionic acid, N-acetylproline, allisin, gentisic acid, 3-hydroxyanthranilic acid, γ-aminobutyric acid and pyroglutamic acid were observed in the early lung cancer group, which is consistent with previous studies [42][43]. This may be due to protein deficiency and increased amino acid requirements caused by tumor growth [44]. According to Takamori S. et al. [45], profiles of 10 salivary metabolites differed markedly between lung cancer and benign lung lesion patients. Among them, salivary tryptophan concentration was significantly lower in patients with lung cancer. It is known that the level of tryptophan in the blood serum in patients with lung cancer was significantly

lower than in healthy people [46]. Takamori S. et al. [45] developed an MLR model in which four metabolites were selected, including lysine and tyrosine. The MLR model had a high ability to distinguish between lung cancer and benign lung lesion patients (AUC = 0.729**)**. It should be noted that in lung cancer, in both studies, the content of amino acids was downregulated.

Glioblastoma. Garcia-Villaescusa A. et al. [47] showed that four amino acids leucine, isoleucine, alanine, and valine change their content in glioblastoma. It is known that alanine is elevated in malignant brain tumors and, therefore, can be used to distinguish between tumor type and grade [48]. In addition, an increase in leucine-rich proteins was observed in some malignant gliomas, which was mainly associated with an increased risk of developing astrocytomas and glioblastomas [49]. A study by Bark J.M. et al. [50] identified valine among a large number of metabolites, which overlaps with the results of García-Villaescusa A. et al. [47] and other studies showing the contribution of valine to cancer diagnosis.

Thyroid cancer. The only study of amino acids in saliva in thyroid cancer [51]. The authors showed that the content of all studied amino acids in thyroid cancer is reduced compared to healthy controls. The results are consistent with previous blood metabolism studies for the detection of thyroid cancer. Abooshahab et al. described a marked decrease in the concentrations of valine, phenylalanine, proline, glycine, methionine and threonine in patients with thyroid cancer [52]. Huang et al. reported in a comparative study between healthy controls and thyroid cancer that the expression of alanine, proline and tryptophan was reduced in the patient group [53]. Threshold values have been established that can be used for diagnosis. The values of sensitivity and specificity for individual amino acids vary within a fairly wide range, the average sensitivity was 76.9% (50.8–100.0%), and the average specificity was 55.1% (43.1–92.2%). When using the complex index, the values of sensitivity and specificity increased significantly and amounted to 91.2 and 85.2%, respectively.

Colorectal Cancer. In the only study by Kuwabara H. et al. [54] showed that several amino acids, such as isoleucine, valine, lysine, and alanine, were elevated in both adenomas and colorectal cancer, but the authors do not offer justification for what this may be due to.

Hepatocellular cancer. Hershberger C.E. et al. [55] proposed four variants of algorithms for detecting hepatocellular cancer based on metabolomic profiling of saliva, but only one of the algorithms included the amino acids glutamine and serine. It has previously been reported that serum serine levels are altered in patients with cirrhosis compared to healthy individuals and in the urine of patients with hepatocellular cancer compared to healthy individuals [56][57]. Glutamine levels differed in serum and liver tissue between healthy people and people with cirrhosis, healthy people and people with hepatocellular cancer [58][59]. The enzyme responsible for glutamine production, glutamine synthetase, has been identified as a potential biomarker for early hepatocellular cancer in proteomic assays and has been shown to promote cell migration by mediating epithelial–mesenchymal transition [60].

Pancreas cancer. Sugimoto M. et al. [24] identified two amino acids in five metabolites for diagnosing pancreatic cancer: phenylalanine and tryptophan. Although pancreatic cancer samples showed a trend towards decreasing

levels of amino acids, including leucine, isoleucine, valine, and alanine [61], an increase in amino acid levels was observed in saliva. In this regard, the authors argue that further validation of these results by comparing saliva profiles with blood and tissue profiles is necessary in order to understand the reason for the different amino acid profiles in saliva.

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