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POLYAMINES AND GAMMA-SYNUCLEIN AS POSSIBLE BIOMARKERS OF THE MALIGNANCY DEGREE OF PAPILLOMAS. POSSIBILITIES OF THE COMBINED USE OF "DFMO" AND "ARMENICUM" AS EFFECTIVE MEANS FOR SYMPTOMATIC THERAPY OF PAPILLOMAS

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Abstract

At present, a subject of a broad discussion are aspects related to the search of biomarkers for assessing the severity of many malignant and benign neoplasms, and more specifically as informative criteria for their early diagnosis and prognosis. The tactics for palliative treatment of malignant diseases, as is known, is based on clinical and laboratory test systems in which specific biomarkers are pathognomic for each disease.

At the same time, such biomarkers are not specified for papillomas of various localization and severity degree. This circumstance, first of all, concerns the papillomas of internal organs (larynx, breast, prostate, cervix), especially when they are classified as papillomas of high severity, with a tendency of their malignancy.

In this publication, the subject of discussion are literary sources, in which, the possible role of aliphatic polyamines – putrescine, spermidine and spermine, is considered as diagnostic and prognostic criteria for the appearance of papillomas in internal organs. According to the authors of this review article, shifts in the content of polyamines in plasma and erythrocytes and in the papillomatous cells can be considered as criteria for the degree of transformation risk of benign cells into malignant. Moreover, the subject of special study should be the researches aimed at clarifying the role of γ-synucleins in the process of papillomatous cell malignancy and the search for new means of symptomatic therapy of high-risk papillomas.

Keywords: *papillomas, malignant neoplasms, γ-synuclein, α-difluoromethyl-ornithine, Armenicum.*

Cancer, papillomas and human papillomavirus

Papillomas are pathological formations on human body, which are subdivided into two types – genital and squamous, and appear as verrucas and genital warts. Papillomas can occur anywhere on the body. Papillomas are q1uite often found in a number of internal organs: on skin, in breast, cervix, and organs of oral cavity.

Papillomas of viral etiology are considered as an oncological disease. Viral infection is transmitted only from person to person, i.e. papilloviruses are purely anthroponotic pathogens. In humans, papillomaviruses are initially localized in the basal layer of epithelium, especially in the areas of metaplasia of the stratified squamous epithelium in the cylindrical. In the epithelial cell cytoplasm, the virus exists in two forms – episomal (outside the chromosome) and intrasomal, integrated into the cell genome [*Munger K et al., 2004*]. Episomal form is characteristic of benign papillomas, and intrasomal – for malignant ones. In carcinomas of different localization, the virus exists in an integrated form, previously localized in the papilloma of the cervix. [*Bosch F et al., 2002; Munger K et al., 2004; Doorbar J et al., 2012*].

The first information about the connection of

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the body infected with papillovirus with malignant neoplasms of the cervix (cervical cancer) appeared in the past years of the last century. In recent years, schemes for the conservative treatment and prevention of cervical cancer have been widely developed, using drugs of different nature and activity [*Vlastos A, 2003*]. Considering the fact that, low-risk and high-oncogenic papilloviruses are the main etiological factor of induction of genital warts, cervical neoplasia with varying degrees of damage (mild, moderate, severe), the main drugs used in oncology were selectively directed at suppressing viral activity. In addition, the search of biomarkers for the purpose of their use in the chemoprophylaxis of cervical cancer also serves as a subject of separate study to this day [*Nishioka K. et al., 1995; Melgarejo AB, Lyon RR, Mitchell MF*. Polyamines as biomarkers of cervical intraepithelial neoplasia. J Cell Biochem Suppl. 1995*Vlastos A, 2003; Bassir H et al., 2015*].

Thus, in a highly informative report of Vlastos A co-authors (2003), such compounds as beta-carotene, follet, vitamins A, C and E, retinoids were used as biomarkers. As Vlastos A. and co-authors rightly point out, "Since the human papillomavirus is the main etiological agent, the tested drugs must be active against the human papillomavirus (HPV) – preinvasive and invasive cell lines" [*Lee S.H. et al., 2003; Vlastos AT et al.,* 2003].

There is no doubt that by the term "pre-invasive cell lines," the authors implied neoplastic degeneration of normal epithelium into papillomas.

According to authors, such drugs, in addition to their selective efficacy on papillomatous target cells, must be non-toxic (or at least have low toxicity), since they must be used over a long period of time [*Vlastos A, 2003*].

Papillomas and polyamines

There are very informative studies in which, in a number of benign and malignant neoplasms, the subject of special studies are processes leading to disruption of the exchange of aliphatic polyamines – putrescine, spermidine and spermine. In connection with the extensive discussion on the role of polyamines in the induction of neoplastic processes, in our opinion, the studies by, published in 1998, deserve special attention [*Boone C et al., 1990; Kelloff G et al.,1996; Soler A et al., 1998*; *Laukaitis C, Gerner E, 2011*]

To clarify the role of aliphatic polyamines in the induction of cutaneous papillomas, the authors put forward specific tasks that they themselves resolved in the course of performing experimental studies. Among the raised questions were the followings:

Are elevated levels of polyamines necessary for the maintenance of papillomatous cell phenotype?

What are the levels of polyamines in normal epithelial cells and papillomatous cells and in situ proliferation processes associated with them?

Which of the three aliphatic polyamines – putrescine, spermidine or spermine, is responsible for the regulatory processes of development and/or maintenance of the papilloma phenotype?

The authors established the important role of polyamines (especially putrescine) in the induction of cutaneous papillomas on model experiments using nonlinear and transgenic mice.

Thanks to the carried out biochemical studies using high-performance liquid chromatography, the authors were able to establish that the levels of polyamines (especially putrescine) in tumor cells are much higher than in adjacent "peripherally" oriented normal epithelial cells. According to them, high concentrations of in situ produced polyamines greatly potentiate the proliferative processes in the so-called stem cells of the basal layer of skin epidermis [*Boone C et al., 1990; Kelloff G et al.,1996; Laukaitis, C, Gerner E, 2011; Akgul M. et al., 2019*].

Due to the conducted research, the authors come to a very important, in our opinion, conclusion that putrescine is a "cryptogenic polyamine" that regulates the phenotype of papilloma cells. Thus, a high level of in situ produced putrescine can potentiate activating ras mutations that occur under the conditions of using the known carcinogen – DMBA. In this particular case, the authors view the high level of putrescine as an "induced oncogene".

There is a considerable amount of information about the role of aliphatic polyamines in the induction of papilloma in numerous internal organs and tissues. A number of papillomas are determined to be of viral origin. The aspects associated with subtle enzymatic mechanisms which ensure the inclusion of polyamines in normal and hyperplastic cells and the interaction of the same enzymatic processes with the human papilloma

virus types 18 (HPV 18) are discussed in a highly informative study of a number of authors [*Jeon J et al., 2003*]. The studies have been conducted in vitro and in vivo. The target of enzymatic research was to the study of transglutaminase 2 (T Gas 2) i.e. one of enzyme families which "catalyzes protein modification by means of polyamine introduction into substrates and the formation of protein crosslinks". Transaminase 2 has been found to interact with oncoprotein E7 of human papillomavirus type 18 (HPV 18). At the same time, according to the authors, the capability of this enzyme to inactivate another HPV16E7 type of human papillomavirus indicates the high prevalence of HPV16 in cervical cancer.

There are also some researches of a considerable interest, aimed to study aliphatic polyamines as risk factors in inducing cervical cancer associated with sexual behavior. Thus, Fletcher S. and co-authors (1991) studied the effect of aliphatic polyamines, identified in seminal fluid, upon the cell cycle as well as ploidy of cervical cells and "primary" epithelial cells, cultured from cervical biopsy samples. The cell growth processes were not impaired, however, in a number of samples, signs of hypodiploidy or hyperdiploidy were observed, depending on the concentration of spermine and spermidine in the seminal fluid. The authors believe that there is an interaction between polyamines of seminal fluid and the DNA of uterine mucous cells, which leads to the ploidy changes, which is often fraught with the development of dysplasia. Clinicians and first of all oncologists and sexopathologists should pay special attention to the fact that the pathogenesis of cervical cancer is conditioned not only by the persistence of the human papilloma virus in situ. It is a known fact that seminal fluid is relatively rich in putrescine, spermidine and spermine. A number of authors [*Fernandez C et al., 1995*] attempted to identify the role of seminal fluid polyamines as cofactors in the development of cervical cancer. The researches of many authors are based on the well-known fact, that aliphatic polyamines (putrescine, spermidine and spermine) undergo oxidation by polyamine oxidase and diamine oxidase, with the formation of oxygen radicals, hydrogen peroxide and reactive aldehydes, which apparently can cause cytotoxic, mutagenic and immunosuppressive in situ effects on the cervical mucosa. According to the results, the authors came to a quite reasonable conclusion that high levels of polyamine oxidase and diamine oxidase in the cervical mucosa should be considered as increased risk factor for cervical cancer, especially in cases with high concentrations of putrescine spermidine and spermine detected in the seminal fluid. At the same time, some authors are quite right to assume that "papilloma-virus infection might synergize the effects of polyamine oxidation, suppressing apoptosis in uterine mucosa cells, carrying potentially oncogenic mutations, which leads to the survival and proliferation of transformed cervical cells", in other words, resulting in their malignancy.

Some aspects concerning the role of aliphatic polyamines in the origin of papilloma are also presented in the studies [*Koza R et al., 1991*]. During the experiment, the authors induced epidermal papilloma in mice DM-1 by a single dimethylbenzanthracene application. It was found, that simultaneously with an increase in the activity of ornithine decarboxylase, the levels of putrescine and spermidine in the same neoplastic tissue sharply increase in papilloma (compared to the control indicators). As the authors note, despite the fact that the epidermis normally contains a large amount of ornithine, the level of the latter in the reborn epidermis with papilloma, exceeds the original by 70 times. According to the authors, in case of the papilloma development, control over local processes ensuring the biosynthesis of specific polyamines is lost, as a result of which the processes of proliferation and differentiation of epithelial tissue are impaired. Some authors determined the polyamine content and ornithine decarboxylase activity in the colon mucosa with adenomatous polyps and without in situ hyperplastic process development [*McGarrity T et al., 1990*]. In biopsy specimens, obtained by colonoscopy with the presence of adenomatous polyps in the mucosa, the content of putrescine increased markedly and at the same time high ornithine decarboxylase activity was determined (compared to the control group). Approximately the same indicators were determined in the rectum mucosa in both groups. The authors believe that in situ detection of aliphatic polyamine (putrescine, spermidine and spermine) levels and ornithine decarboxylase activity i.e. in biopsy specimens of the mucous mem-

brane of the rectum should be considered as informative criteria to determine the course of adenomatous polyps of the mucous colon and their malignancy risk degree. A number of researchers [*Mitchell M et al., 1997*] determined the levels of polyamines and ornithine decarboxylase activity in the cervix with its specific areas as a choice for biopsy: in the mucosa, subject to intraepithelial neoplasia and adjacent regions where epithelial cell structure was relatively preserved, i.e. did not morphologically differ from that characteristic of normal mucosa. It was found that indicators of the polyamine levels – putrescine, spermidine and spermine and the activity of ornithine decarboxylase in the regions of epithelial neoplasia were much higher than in adjacent areas, in which epithelial cell structure was preserved. There are informative data on the role of aliphatic polyamines and ornithine decarboxylase in the induction of benign and malignant tumors of the pancreas [*Dunzendrofer U, Russel D, 1978; Feuer E et al., 1999; Simoneau A et al., 2001*]. In pancreatic cancer the level of aliphatic polyamines – putrescine, spermidine and spermine in the pancreas is undoubtedly very high. Moreover, it is known that ornithine decarboxylase is overexpressed in prostate tissue [*Simoneau A et al., 2001*]. It is noteworthy that even in benign prostate diseases, the aliphatic polyamine content and the activity of ornithine decarboxylase are much higher than in benign tumors localized in other organs [*Dunzendrofer U, Russel D, 1978; Devens B et al., 2000*].

Ornithine decarboxylase in mammals acts as the only "launcher" enzyme for the formation of putrescine from ornithine, as indicated previously. That is why the researches in practical oncology, aimed to find new effective means of inhibiting the activity of ornithine decarboxylase [*Metcalf B et al., 1978*] appeared to be the most productive. According to Meyskens F.L. (1999) the most effective blocker of this class of drugs is the "irreversible" ornithine decarboxylase inhibitor i.e. α-difluoromethyl-ornithine (DFMO).

Wallace H.M. (2009) notes that the twenty-year experience in DFMO testing was a precondition for searching and developing polyamine analogues in oncology that should compete with endogenous polyamines (putrescine, spermidine and spermine) by inhibiting the activity and biosynthesis of ornithine decarboxylase. The studies of Boiko I. V. and co-authors (1998) on the therapeutic efficacy of DFMO in the "treatment" of cervical intraepithelial neoplasia are, in our opinion, of considerable interest. Boiko I. V. studied the effect of DFMO on the expression of epidermal growth factor, which is known to be a marker of cervical intraepithelial neoplasia progression. As shown in the research results, the localization of epidermal growth factor is limited to the basal layer of the epidermis in the normal (control) epithelium, whereas in case of cervical intraepithelial neoplasia, the expression of epidermal growth factor was more common and spread to other, more superficial layers of the epidermis. The DFMO application for therapeutic purposes in cervical intraepithelial neoplasia limited significantly the distribution of epithelial receptors to the epidermal growth factor. This allowed the authors to conclude that the progression of cervical neoplasia is associated with spatial dysregulation of the epidermal growth factor, which can be reversed by using DFMO. The considerable therapeutic efficacy of DFMO was revealed in the researches of a number of authors [*Mitchell M et al.,1998*] in complex therapy of the patients with grade III cervical intraepithelial neoplasia. The use of DFMO was accompanied by decreased spemidine/spermine tissue index and increased level of ornithine in the plasma of the patients with cervical neoplasia.

PAPILLOMAS AND γ-SYNUCLEIN

Over the past 20-25 years, in addition to a number of malignant tumors, the subject of widespread discussion is the detection of γ-synucleins in the cytoplasm of tumor cells, as well as in plasma and erythrocytes.

A number of authors have shown that the expression of γ-synuclein in tumor cells significantly stimulates the process of proliferation and invasion, as well as metastasis of breast cancer cells [*Liu Y et al., 2000; Lu A et al., 2002; Jiang Y et al., 2003; 2004; Singh V, 2008; Droog M et al., 2016*].

In particular, it was shown that in linear animals the overexpression of γ-synuclein exhibits the ability to stimulate tumors, migration to lymph nodes and lungs, unlike γ-synuclein-negative cells [*Jia T et al., 1999*].

It should be noted that in the available literature it was not possible to find out exhaustive information concerning the presence of oligomeric

and aggregated γ-synucleins in neoplastic cells of papillomas of various localization. Meanwhile, it's not excluded that the "cumulation" of γ-synucleins in papilloma cells, along with a high level of polyamines in the same neoplastic cells, can serve as additional, but at the same time, very informative criteria for chemoprophylaxis of papillomas, and possibly assess the transformation risk of papillomatous benign cells into malignant [*Kreider JW, Breedi C., 1969*].

With this aim, over the past 5 years, many thin biochemical, immunofermental and immunomorphological studies have been carried out in the laboratories of morphology and enzyme immunoassays of the Scientific Research Center (SRC) of the Yerevan State Medical University (YSMU) after M. Heratsi. Those studies were conducted on laboratory animals that reproduced papilloma models according to the adopted schemes [*Gómez-Cuadrado L et al., 2017*]. The study results will be summarized and published in the open access journal.

Treatment tactics for papillomas

Currently, "Armenicum" LLC has launched a serial production of a therapeutic agent – "Armenicum", which in various dosage forms has been successfully used in the treatment of HIV infection, hepatitis C, systemic lupus erythematosus, and wound healing. The drug has a pronounced antiviral, antibacterial and anti-inflammatory spectrum of action. It is not excluded that "Armenicum", due to its antiviral spectrum of action, can be an effective means of preventing the development of the neoplastic process in papillomas.

It should be noted that various "Armenicum" dosage forms were the subject of special biochemical, immunofermental, immunomorphological and pharmacological studies of YSMU SRC staff and "Arpimed" Limited Liability Company (LLC), in which it was very convincingly shown that the paste and ointment "Armenicum" have a pronounced antibacterial and anti-inflammatory effect on the course of purulent wound process, at the same time preventing the development of hyperplastic processes in composite components of skin [*Zilfyan A et al., 2016*].

That is why the employees of both groups will test the "Armenicum" dosage form on the induced papilloma model, in order to summarize the preclinical study results, for its subsequent introduction into practical oncology, as a chemoprophylactic agent for treating papillomas.

According to literary sources, most researchers state the positive effect of DFMO, which is used in the treatment of a number of malignant neoplasms; either isolated or in combination with other antitumor agents.

At the same time, there are also a number of very informative, but opposite directions, information, according to which the use of DFMO in oncological practice is not very effective [*Boone C et al., 1990; Kelloff G et al.,1996; Laukaitis C, Gerner E, 2011; Bassir H et al., 2015*].

In this regard, it is necessary, in our opinion, to pay attention to the following circumstance. As a rule, the use of DFMO was carried out on oncological patients in a relatively late stages of malignant tumor development, i.e. in the period when the tumor tissue almost looks like formed and has a high degree of risk of metastasis. In case of a radical removal of the tumor and the use of highly toxic cytostatics of a selective spectrum, in addition to metastases, relapses occur quite often. That is why, as a number of authors rightly point out, the use of DFMO in specific oncological situations is ineffective.

In our opinion, if aliphatic polyamines and γ-synuclein begin to appear as possible factors interested in the induction of malignant tumors, then in these patients, on the background of high polyamine levels in blood and in tumor cells themselves, the synthesis and intracellular aggregation of γ-synucleins are already at the completion stage.

In our opinion, the primary important task is to choose another methodological approach based on determining the levels of polyamines and γ-synuclein at the earliest stages of malignant growth, i.e. in advance – before the appearance of "atypical proteins" like γ-synucleins in tumor cells. In addition, a certain contingent of patients with benign tumors should serve as the subject of special clinical and laboratory studies. In this particular case, it is necessary to conduct thin laboratory studies (high-performance liquid chromatography, ELISA and immunomorphological analysis) for the determination of polyamines and γ-synucleins in blood plasma and erythrocytes, as informative criteria for assessing the risk of malig-

nancy of a previously benign tumor.

It is possible that such methodological approach, based on the choice of a specific contiguant of individuals with benign and malignant neoplasms, will be more acceptable in terms of the therapy with DFMO.

Based on the analysis of literary sources, we can come to the following conclusion:

It is necessary to conduct thin biochemical immunoassay and immunomorphological studies on clinical and experimental material to determine the levels of aliphatic polyamines – putrescine, spermidine, spermine in plasma and erythrocytes of blood, as well as neoplasticly reborn papilloma cells.

In the dynamics of cutaneous papilloma development (on experimental material) and in case of radical (surgical) removal of papillomas, it is necessary to determine the presence of γ-synucleins in the resected tissue (ELISA and immunomorphological analysis).

Based on the obtained results (see pp. 1 and 2), to carry out a correlation analysis between shifts in the content of polyamines in the studied biological objects and the presence of γ-synucleins in the same biological objects.

If a direct positive correlation is established between a high level of aliphatic polyamines and the presence of γ-synucleins in the same papillomatous cells, they should be ranked as biomarkers characterizing the developmental characteristics and degree and nature of the malignancy risk of papilloma cells.

Under the conditions of an experimentally induced model of cutaneous papillomas, it is necessary to determine the effectiveness of the combined use of DFMO and "Armenicum" – as symptomatic agents that inhibit the growth of papillomatous cells and, possibly, their transformation into malignant ones.

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