Molecular characteristics of amitozyn antitumor action

L.P. Shvachko, I.G. Bukh, I.V. Alekseeva, A.I. Potopalsky

Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine

Amitozyn – an antitumor phytogenous preparation derivates from cumulative alkaloids of celandine alkylated by thiophosphamide in accordance with original elaboration of A.I. Potopalsky at the Institute of Molecular Biology and Genetics of NASU [1].

Clinical trials determine Amitozyn antitumor action on the early stages of oncological diseases with considerable immunomodulatory effect [2]. An interesting fact was specified: amitozyn enables the organism to normal co-existence with tumor during a long period of time, thus, it is able to inhibit tumor cells growth and to prevent recurrence after tumor removal. This unusual amitozyn property not only requires the researches of molecular basis of the preparation antitumor action, but also testifies and proves the possibility of the molecular ante-cancer mechanism existence in the tumor dynamic progress. Somatic mutagenesis conception of carcinogenic type in the induction of malignant cell transformation is matched, first of all, both with etiology of clonal somatic derivation of sporadic tumors, and with recognition of oncoprogression as genome "disease" with a long latent period before tumor appears. Study of molecular specificity of ante-cancer mechanism on the somatic mutagenesis level of carcinogenic type and knowledge of the early adequate targets in oncological process induction clear the ways of forecasting and early prevention of the malignant cell transformation development.

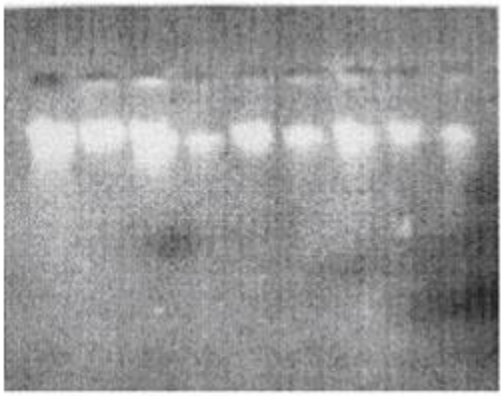
Convincing molecular mechanisms of carcinogenesis are in the dedifferentiation malignant cells area that probably associates with damage of epigene control, where the mechanism of DNA methylation attracts attention [3]. Action of the specific inhibitors DNA methylation antimetabolites by cytosine residues consisting of CpG dinucleotides – 5-aza-cytidine and 5-deoxy-aza-cytidine, testify key role (pattern) of DNA methylation in inactivation of transcription and organization of transcriptionally silent (up to 95%) genome state and gene expression [4]. Along with this fact, embryonic lethal mutations

of the DNA-methyltransferase key gene (pattern) show embryogenesis and embryonic cells differentiation direct dependence from DNA methylation state of genome [5]. It should be noticed that phenomenon of epigene DNA hypermethylation of 5'-nonmethylated CpG islands in oncosuppressor gene promoter areas, discovered for the recent years, and, as a result, expression inhibiting of these genome protection key genes during the analyses of the vast majority of tumors and tumor cell lines, recently is the main issue in de novo DNA methylation and carcinogenesis mechanism combination. However, we showed that on somatic mutagenesis level of carcinogenic type, at the analysis of peripheral blood lymphocytes of the patient with different tumor progression etiology, there is exactly genome (pattern) DNA-hypomethylation at the expense of significant demethylation (Alu) of satellite DNA repeats (figure 1). Concentrated with methyl-CpG denucleotides (on 56%) and the most numerous, consisting about 10 % of genome [7], Alu repeats are probable molecular sensors of DNA methylation state on the level of somatic mutative process, related to carcinogenesis induction. We revealed that ante-cancer mechanism phenomenon of tumor progression are in the functional combining of DNA-hypomethylation pattern, demethylation of Alu alpha satellite repeats and centromeric/pericentromeric heterochromatin decondensation, the result of which is centrometric metaphase chromosomes instability with premature sister chromatids separation and anomalous chromosomes segregation on mitosis stage [8, 9].

Figure 1.

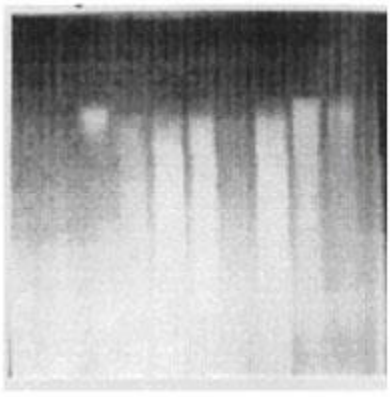
DNA state of hypomethylation at the tumor progression on the base of Hpa II-sensible to nonmethylated CpG sites of epdonuclease restriction.

1a **Control**



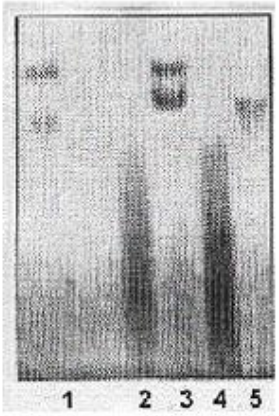
1-9 – genome DNA
of healthy donors

1b **Tumor progression**



1 – control – DNA of λ phage
2 – embryonic DNA
3 – DNA of the patient with thyroid gland cancer
4 – DNA of the patient with colorectal cancer
5 – DNA of the patient with neuroblastoma
6 – DNA of the patient with Wilms' tumor

1c **Molecular hybridization DIG – (Alu) of satellite DNA repeats with profile of DNA hypomethylation at the tumor progression.**

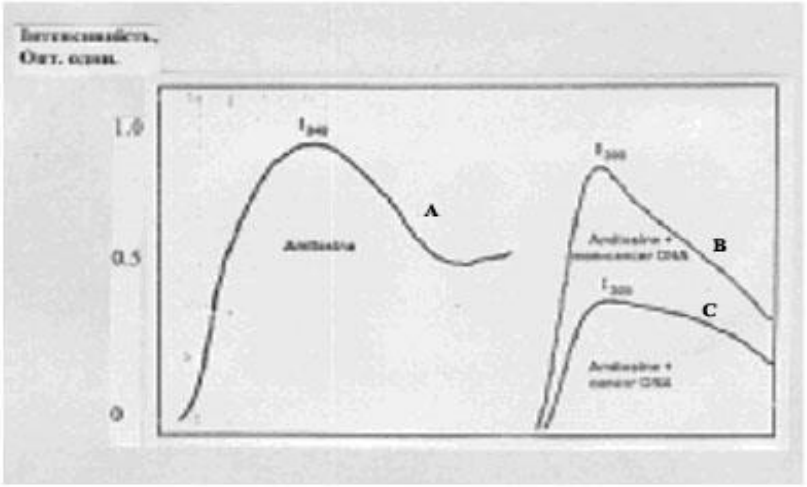


1- DNA of healthy donor
2- Embryonic DNA
3- Cancer-associated DNA

We studied Amitozyn, as an antitumor preparation, in respect to DNA (pattern) hypomethylation phenomenon in prevention of the tumor progression induction on ante-cancer mechanism level. As amitozyn includes alkaloids with high fluorescence (berberin, chelidonin, sanguinarin), auto fluorescence is inherent for the preparation and underlies in the specificity of preparation action [2, 10]. Thus, according to fluorescence spectra at the wave length 300-340 nm, amitozyn differentiation specificity in genome DNA-binding of the lymphocyte cells at the tumor progression and normal DNA of lymphocytes, at the heart of which – principal changes in DNA methylation was shown (figure 2).

Figure 2.

Fluorescence spectrum of Amitozyn (A) with native preparations of genome DNA of healthy donor (B) and at the tumor progression (C).

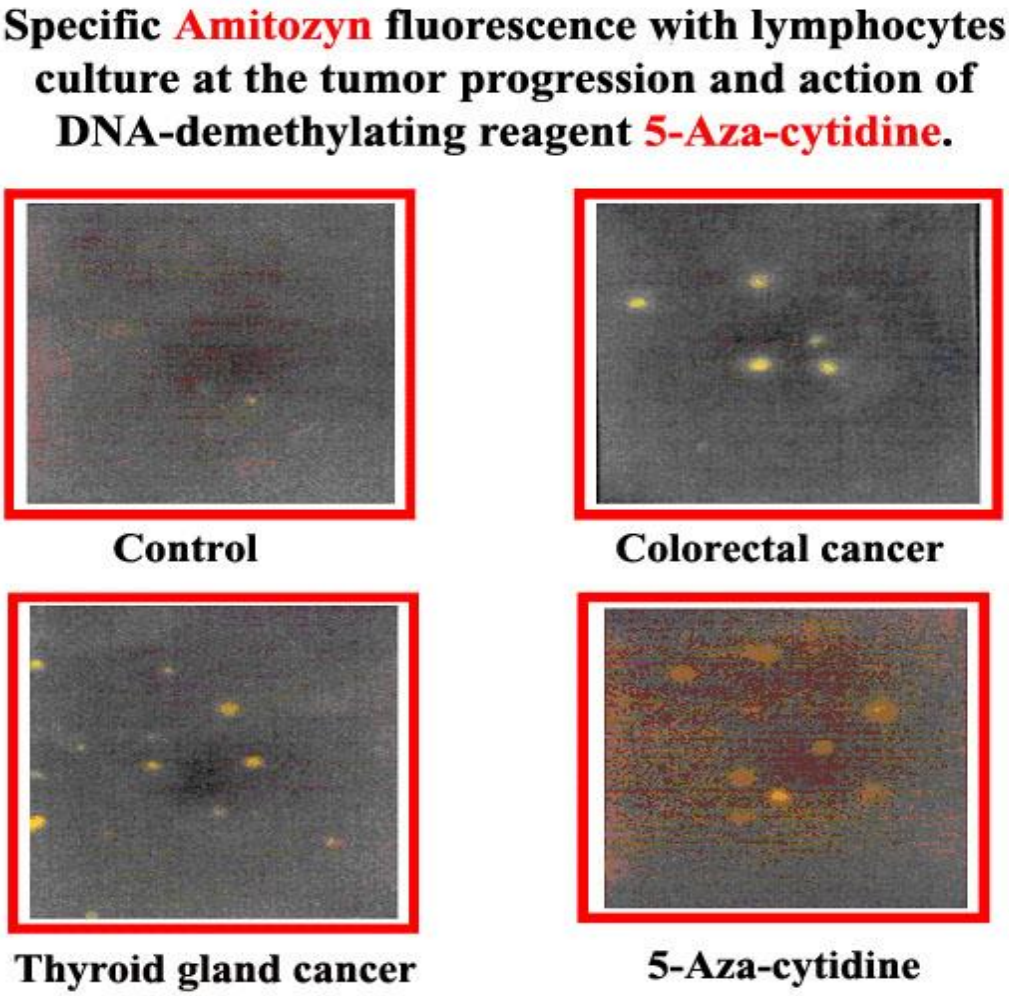


A - amitozyn (25 mkg/ml).
B, C – DNA (10 mkg/ml) + Amitozyn ($4+10^{5}$)

Further researches can be also directed on analysis of the tumor and non-tumor DNA conformational characteristics with amitozyn as a specific ligand. According to the clinical researches of Potopalsky and co-authors [10], specific fluorescence of amitozyn adsorption (2-4 hours after introduction to the patient) was revealed exactly on the tumor tissue and it was absent on the healthy tissues remote from the tumor that demonstrates amitozyn connection to molecular mechanism of tumor progression, trigger stage of which, in our opinion, is an ante-cancer mechanism connected with DNA

hypomethylation. We showed that amitozyn selective molecular sensibility to pattern DNA hypomethylation phenomenon took place at specific fluorescence both in lymphocytes culture of the patients with oncological progression, and in population of the healthy lymphocytes, cultivated with DNA-demethylating reagent 5-aza-cytidine at the absence of amitozyn fluorescence in the lymphocytes culture of the healthy donors (figure 3).

Figure 3.

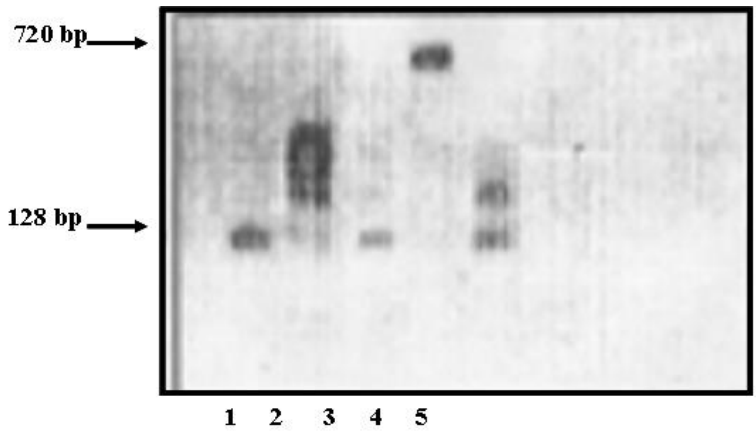


Exactly this fact gives to amitozyn prognostic importance in cells with DNA hypomethylation stage revealing on the level of somatic mutagenesis of carcinogenic type.

5-Aza-citidine is a specific competitive inhibitor of the DNA genome methylation key ferment – (pattern) DNA-methyltransferase [5]. According to the result of polymerase chain reaction (PCR) analysis of DNA-methyltransferase gene, it was shown that oncological progression on the level of somatic blood cells is accompanied by pattern DNA-methyltransferase gene loss or inhibition. It is combined with simultaneous profile appearance of transcripts de novo not only typical, but also in principle essential for the further research of de novo DNA hypomethylation phenomenon at the tumor progression and its coordination with DNA hypomethylation phenomenon. It was shown that amitozyn, as an antitumor preparation, is effective molecular protector in appearance of de novo profile of DNA-methyltransferase gene variants with dominating transcription of pattern DNA-methyltransferase variant in the lymphocyte cells of the patient with oncological progression after amitozyn cultivating during 72 hours (figure 4).

Figure 4.

Profile of DNA genetic polymorphism – methyltransferase gene at the tumor progression according to PCR-amplification results



1 – Genome DNA of the healthy donor; 2 – Genome DNA with oncological progression; 3 – Oncological progression + Amitozyn (lymphocytes culture at amitozyn presence, 72 hours); 4 – Internal PCR – control; 5 – Mitogen-stimulated lymphocytes culture of the healthy donor, 72 hours.

Thus, the research results enable us to state on existence of DNA hypomethylation phenomenon on the level of ante-cancer mechanism of tumor progression as a key factor of somatic mutagenesis of carcinogenic type. It is shown that amitozyn antitumor action, both on the cellular, and on the molecular levels, specifically joins genome DNA hypomethylation protection mechanism and can simultaneously accumulate prognostic, diagnostic and medicinal functions of the antitumor preparation of molecular action.

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