MICROBIOTA ON THE SURFACE OF MELANOMA MAY PROMOTE IN DERMAL INVASION OF MELANOMA

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Abstract

It is not yet known exactly what happens first: tumor invasion and the associated change in the microbiota, or, conversely, changes in the microbiota before tumor invasion.

The aim of the work was to investigate the contamination and colonization of microbes in melanoma of the skin.

In 23 primary melanoma patients the samples for microbiological examination were taken from melanoma surfaces and from non-lesioned skin before surgery. Microorganisms were cultured at optimal temperatures on elective or differential media appropriate for each taxon and were identified by bacterial analyzer. After wide local excision of melanoma the histological examination determined Breslow thickness and Clark's level of melanoma invasion; Loeffler's methylene blue staining was used to detect the colonies of microorganisms.

From intact skin 62 bacterial cultures were isolated with density of colonization in 1.2×10^3 – 6.4×10^3 CFU/cm^2. From ulcerated surface of melanoma 25 bacterial cultures were identified. The concentration of microorganisms was significantly higher on ulcerated melanomas. The colonization density of S. aureus was highest; its concentration was 5.8×10^7 comparing to 6.4×10^3 CFU/cm^2 on intact skin. Concentration of gram-negative rods was high also; e.g: E. coli and P. putida were 6.2×10^6 and 1.8×10^5 CFU/cm^2 respectively. Loeffler's staining of histological specimens revealed colonies of microorganisms at the bottom of melanoma ulcers. In case of ulcerated melanoma with Clark level IV invasion the microbial colonies were identified in the reticular dermis.

The spectrum of microorganisms on the surface of intact skin is twice as large (62 vs. 25) as on surface of ulcerative melanoma, but the concentration of microorganisms is significantly higher on ulcer tumor's (10^5–10^7) surface than intact skin (10^3–10^4).

Microbiota on the surface of the chronic ulcer may increase local pathogenicity leading to tissue degradation that may be essential for intradermal melanoma invasion.

Keywords: skin melanoma, microbiota, microbial colonies

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Introduction

Numerous studies have been published highlighting the relationship between gut microbiota and melanoma or other cancers [1–5]. They showed the host microbiome has come to the forefront as a potential modulator of cancer metabolism, and the bacterial biofilms might act as direct triggering factors contributing to cancer [6, 7].

The bacteria that reside specifically within cancer have been found to be tumor-type specific, suggesting an association with cancer development [8, 9].

Recent studies indicate the association between bacteria and the tumor microbiome is complex and remains poorly understood. How the skin microbiota affects melanoma has not yet been studied [10, 11]. However, microbiomes in inflammatory diseases of skin have been suggested to be associated with squamous skin cancer [12]. Salava et al. [13] reported minimal differences in the stratum corneum microbiome between melanoma and benign nevi, although melanoma samples seemed to have slightly less microbiome diversity. In experimental pig melanoma model was found that microbiome diversity in full thickness biopsies from non-lesion skin had a significantly different microbiome from that observed in melanoma tissue [14]. In addition, we must take into account that melanoma cells deeply interact with the tumor microenvironment and the immune system [15, 16]. Overall, further studies to unravel the potential influence of microbial exposure on tumor genesis are highly warranted and might pave a way for novel strategies for cancer therapy [17, 18].

The development of a malignant tumor is accompanied by its invasion and metastasizing, which can also affect the vector of invasion of bacteria contained in the tumor. It is not yet known exactly what happens before: tumor invasion and the associated change in the microbiota, or, conversely, changes in the microbiota induce tumor invasion.

Aim: to investigate the contamination and colonization of microbes in skin melanoma.

Material and methods

This study was approved by the Ethics Committee of the Ternopil National Medical University and conducted according to the principles of the Declaration of Helsinki. All participants were enrolled after providing written Informed Consent. Patients with primary skin melanoma T3–T4 (n=23, average age 52±9 years, 9 male and 14 female) admitted to the Ternopil Regional Clinical Oncology Hospital were included in study.

Samples for microbiological examinations were taken by a cotton-tipped swab from the melanoma surfaces (ulcers) and from the non-lesion skin (at a distance 5-6 cm of the tumor) on 3-4 days before surgery. Transport medium (AMIES) that conserves viability and prevents the growth of microorganisms was used. The skin swab sampling procedure and cultivation of microorganisms were performed in an accordance of the standard rules. Microorganisms were cultured at optimal temperatures on appropriate for each taxon elective or differential media: salt-yolk agar (for staphylococci determination), Endo medium (for enterobacteria and nonfermenting bacteria determination), Thioglycolate broth and sugar blood agar (for anaerobes cultivation), sugar meat-pepton agar (to enumerate colony forming units (CFU)), sheep blood agar (to detect hemolytic activity of microorganisms). Culture was considered positive if concentration of microbes was at least 103 CFU/cm2. Contamination level 105 CFU/cm2 was considered critical, it testified the role of bacteria in development of infectious process and probably in cancerogenesis. Microorganisms were identified by bacterial analyzer Vitek2 Compact (bioMerieux, France).

All patients underwent radical surgery – wide local excision of melanoma. During histological examination, paraffin sections were stained with hematoxylin-eosin to determine the Breslow thickness of tumor and Clark's level of

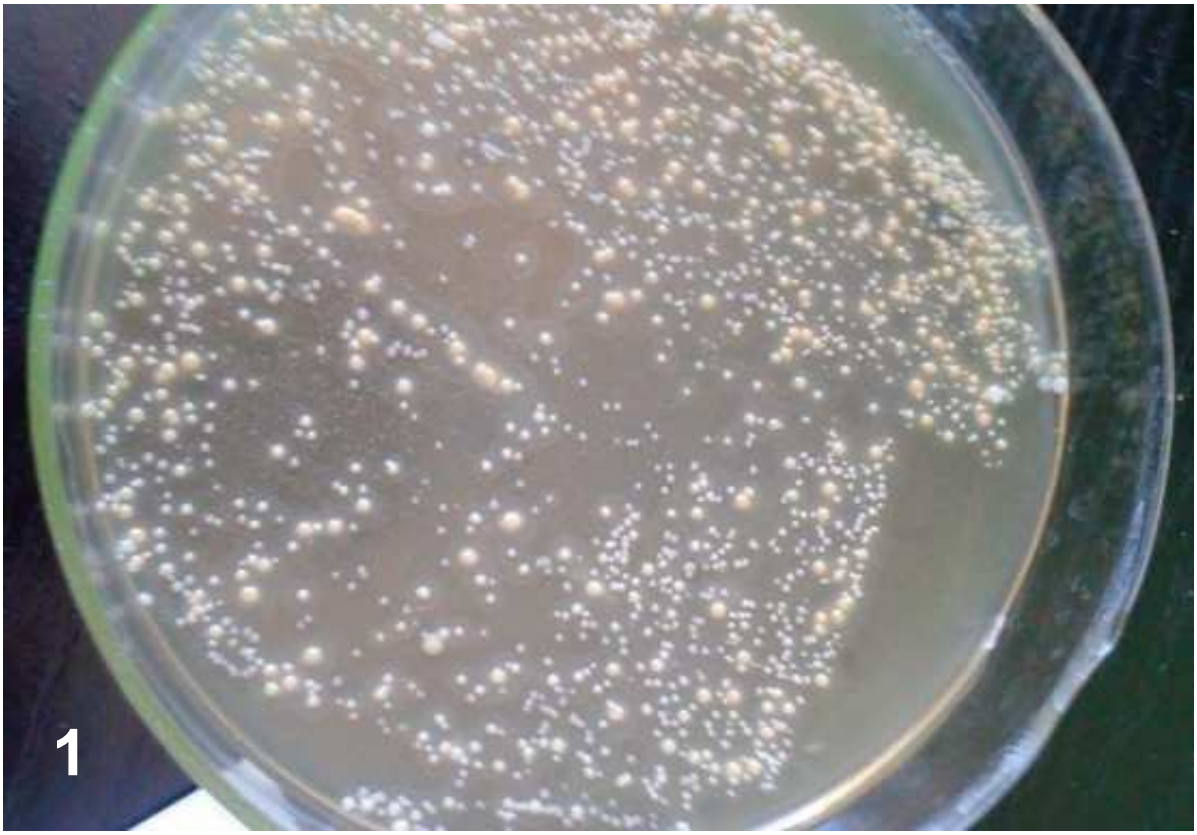
melanoma invasion; the Loeffler's methylene blue staining was used to detect the colonies of microorganisms.

Samples obtained from swabbing provide information on the superficial microbiota composition, whereas morphological specimens of removing melanomas offer the opportunity to study microorganisms that could inhabit the deepest layers of the skin.

Results and Discussion

From intact skin 62 bacterial cultures were isolated. The various species of bacteria were identified, however most of them were in low density and not included in this study. Gram-positive cocci, such as: Staphyloccocus aureus, S. epidermidis S. lentus, S. haemolyticus, Kocuria kristinae, Enterococcus faecalis, gram-positive rods Bacillus megaterium, and gram-negative rods Pseudomonas putida and Acinetobacter baumannii were presented on the intact skin in 1.2×103 – 6.4×103 CFU/cm2 (Table). They were included into 3-component-associations mostly. The frequencies of occurrence of staphylococci were highest on intact skin.

From ulcerated surface of melanoma gram-positive cocci (S. aureus, S. epidermidis) and gram-negative rods, such as: enterobacteria (E. coli, Klebsiella pneumoniae, Proteus vulgaris), and nonfermenting bacteria (A. baumannii, Pseudomonas putida) were identified (n=25). Microbial spectrum was less diverse on melanoma ulcer (Table). Similar data was shown by Mrázek et al. (2019): both bacterial composition and diversity were significantly different between the skin and melanoma microbiota. In our study the frequency of occurrence of gram-positive staphylococci on melanoma ulcer was higher in 1.8 times than same index of gram-negative rods. Similar ratio between frequency of occurrence of gram-positive and gram-negative bacteria was on intact skin. However, the concentration of microorganisms was significantly higher on melanoma ulcer. For example, the colonization density of S. aureus was the highest. Its concentration increased up to 5.8×107 CFU/cm2 compare to 6.4×103 CFU/cm2 on intact skin (Fig. 1).



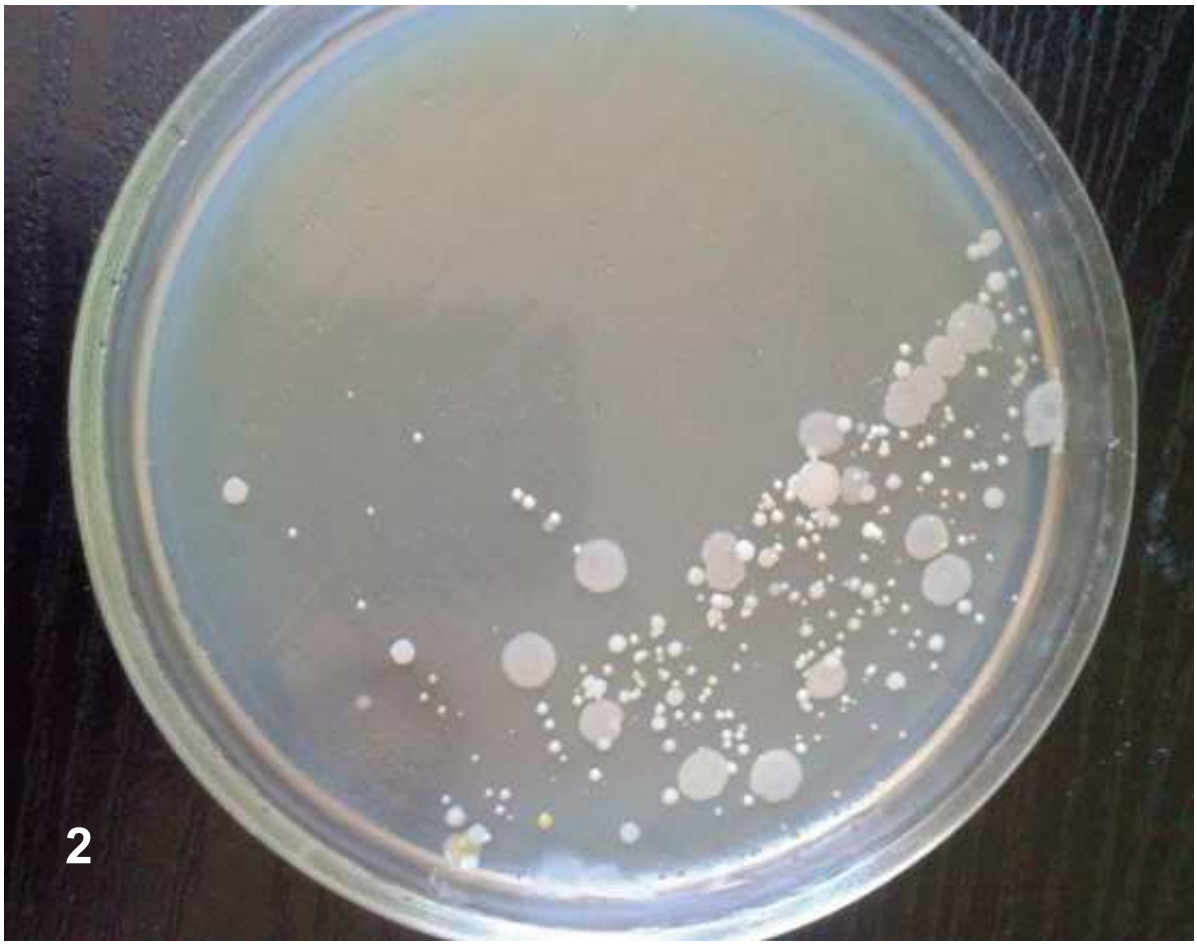


Fig. 1. The growth of bacteria, isolated from ulcerated surface of melanoma (1) and intact skin (2) by streak method, on sugar MPA.

The concentration of gram-negative rods was high also. The P. putida density of colonization was higher in 2 times (1.8×105 CFU/cm2 and 2.4×103 CFU/cm2 respectively).

It is interesting that in the study Squarzanti et al. (2020) was suggested the S. aureus may serve as marker of risk for development of squamous cell carcinoma and contribute to the cutaneous carcinogenesis through the chronic inflammatory state.

Morphological exam of removed melanomas revealed the following depth of tumor invasion: II (4 samples), III (11) and IV (8) level by Clark; 19 melanomas were ulcerated and 4 non-ulcerated. In melanoma without an ulcerative surface and the second level of Clark invasion, single colonies of microorganisms were observed at the level of the papillary dermis (Fig. 2).

In the case of ulcerative melanoma, colonies of microorganisms were determined at the bottom of the ulcer on the background of severe inflammatory infiltration by leukocytes (Fig. 3). In samples of melanoma with ulcer on the surface and the Clark level IV invasion, microbial colonies were identified in the reticular layer of the dermis on the background of lymphocytic infiltration (Fig. 4).

Thus it is the first study to compare microbial contamination on the surface of melanoma and the presence of microbial colonies in the skin, which needs further investigation. A better understanding the role of the microbiota in skin malignancies is necessary, as it could potentially provide further insight into the different roles tissue-specific microbes in cancer progression.

Table1. Microbial diversity and density of colonization of ulcerated surface of melanoma by bacteria compare with intact skin

Microorganism		Ulcerated surface of melanoma		Non-lesion skin	
		frequency of occurrence, %	density of colonization, CFU/cm^2	frequency of occurrence, %	density of colonization, CFU/cm^2
Gram-positive cocci	*K. kristinae*	-	-	34.8	5.6×10^3
	S. aureus	43.5	5.8×10^7	47.8	6.4×10^3
	S. epidermidis	26.1	8.1×10^6	65.2	4.1×10^4
	S. haemolyticus	-		26.1	1.8×10^3
	S. lentus	-	-	17.4	1.2×10^3
	E. faecalis	-	-	8.7	1.4×10^3
Gram-positive rods	*B. megaterium*	-	-	26.1	2.6×10^3
	B. subtilis	-	-	21.7	3.1×10^3
Gram-negative rods	*E. coli*	13.1	6.2×10^6	-	-
	K. pneumoniae	30.4	-	-	-
	P. vulgaris	4.3	3.1×10^5	4.3	1.1×10^3
	P. putida	21.7	1.8×10^5	21.7	2.4×10^3
	A. baumannii	4.3	2.1×10^5	4.3	1.1×10^3

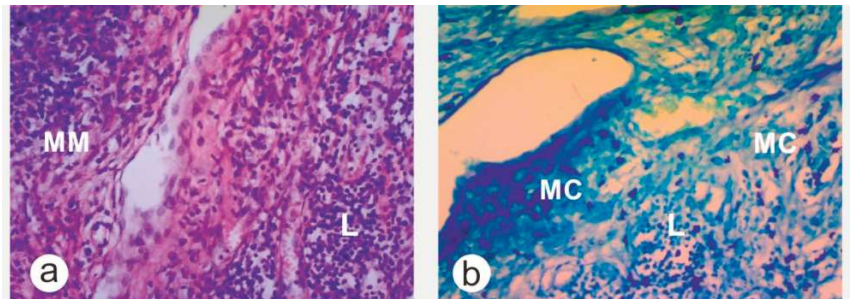


Fig. 2. Histological specimen No. 7908-9. Malignant melanoma (MM) with Clark level II invasion without ulceration: (a) hematoxylin-eosin, ×200; (b) Loeffler staining, ×400; lymphocytic infiltration (L), microbial colony (MC).

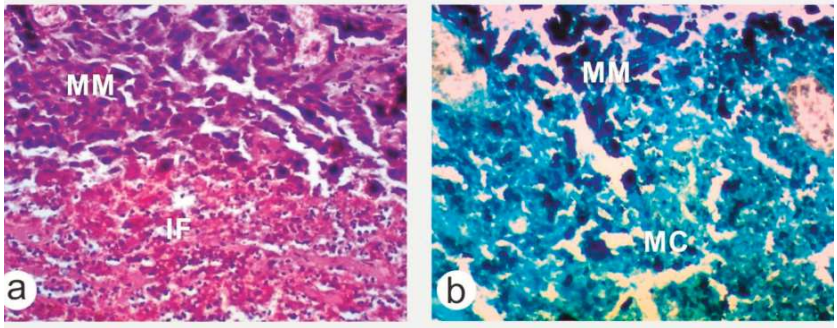


Fig. 3. Histological specimen No. 23082. Malignant melanoma: bottom of ulcer with tissue inflammation (IF), microbial colonies (MC); (a) hematoxylin-eosin, ×200; (b) Loeffler staining, ×200.

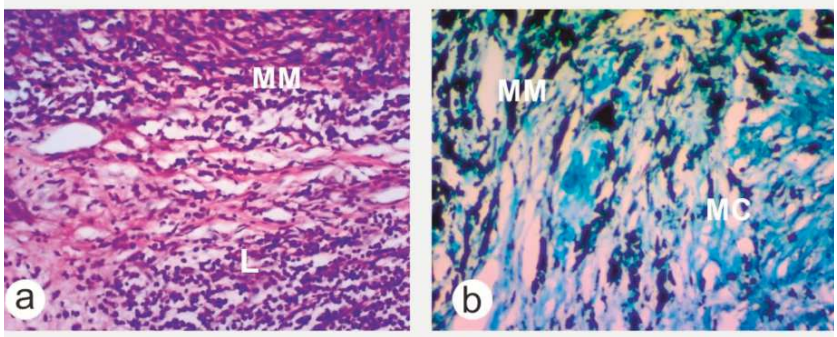


Fig. 4. Histological specimen No. 5550-1. Malignant melanoma (MM) with ulceration and Clark level IV invasion: (a) hematoxylin-eosin, ×200; (b) Loeffler staining, ×200; lymphocytic infiltration (L), microbial colonies (MC) in reticular derma.

Conclusions

Our study shows the qualitative and quantitative differences of microbiota on surfaces of ulcerated melanoma and non-lesion skin. The spectrum of microorganisms on the surface of non-lesion (intact) skin is twice as large (62 vs. 25) as on the surface of ulcerative melanoma, but the concentration of microorganisms is significantly higher on ulcer tumor's (10^5–10^7) surface than intact skin (10^3–10^4).

Microbiota on the surface of the chronic ulcer may increase local pathogenicity leading to tissue degradation that may be essential for intradermal melanoma invasion. With ulcerative melanoma and IV level of its invasion the colonies of microorganisms are located both at the bottom of the malignant ulcer and in the deep layers of the dermis (e.g., reticular).

Resumo

Nun oni ankoraŭ ne scias precize kio okazas antaŭe: tumora invado kaj la rilata ŝanĝo en la mikrobaro, aŭ, male, ŝanĝoj en la mikrobaroj estigas tumoran invadon.

La celo estis esplori la poluadon kaj koloniigon de mikroboj en haŭta melanomo.

En 23 primaraj melanomaj pacientoj la specimenoj por mikrobiologia ekzameno estis prenitaj de melanomaj surfacoj kaj de neleza haŭto antaŭ kirurgio. Mikroorganismoj estis kultivitaj ĉe optimumaj temperaturoj laŭ taŭga elektebla aŭ diferenciala medio por ĉiu taksono kaj estis identigitaj per bakteria analizilo. Post larĝa loka dekoltaĵo de melanomo la histologia ekzameno determinas Breslow-dikecon kaj la nivelon de Clark de melanoma invado; la metilen-blua kolorigo de Loeffler estis uzata por detekti la koloniojn de mikroorganismoj.

De sendifekta haŭto 62 bakteriaj kulturoj estis izolitaj kun denseco de koloniigo en 1.2 × 10^3 - 6.4 × 10^3 CFU/cm^2. De ulcerigita surfaco de melanomo identiĝis 25 bakteriaj kulturoj. La koncentriĝo de mikroorganismoj estis signife pli alta ĉe melanoma ulcero. La kolonia denseco de S. aureus estis la plej alta; ĝia koncentriĝo estis 5,8 × 10^7 kompare al 6,4 × 10^3 CFU/cm^2 sur nerompita haŭto. Koncentriĝo de gramnegativaj baciloj ankaŭ estis alta; ekz: E. coli kaj P. putida estis 6,2 × 10^6 kaj 1,8 × 10^5 CFU/cm^2 respektive. La kolorigo de Loeffler de histologiaj specimenoj malkaŝas la koloniojn de mikroorganismoj ĉe la fundo de melanoma ulcero. En kazo de ulcerigita melanomo kun Clark-nivela IV-invado la mikrobaj kolonioj estis identigitaj en la retoforma dermo.

La spektro de mikroorganismoj sur la surfaco de sendifekta haŭto estas duoble pli granda (62 kontraŭ 25) ol sur surfaco de ulcera melanomo, sed la koncentriĝo de mikroorganismoj estas signife pli alta sur la surfaco de ulcera tumoro (10^5-10^7) ol sendifekta haŭto (10^3-10^4).

Mikrobaro sur la surfaco de la kronika ulcero povas pliigi lokan patogenecon kaŭzante histan degradadon, kiu povas esti esenca por intraderma melanoma invado.

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