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MITOCHONDRIAL DNA MUTATIONS IN PATIENTS WITH HRHPV-RELATED CERVICAL LESIONS

Cristina D. Goia-Rușanu[1]*, Iulia V. Iancu[1], Anca Botezatu[1], Demetra Socolov[2], Irina Huică[1], Adriana Pleșa[1], Gabriela Anton[1]

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ABSTRACT

High risk human papillomaviruses (hr-HPV) are known to be the etiological agents of cervical cancer disease. On the other hand, other cofactors are considered to be important in cervix carcinogenesis. Mutations in mitochondrial DNA (mtDNA) as well as alterations in mtDNA content have been reported in numerous cancers examined to date. The D-loop region has been shown to be a mutational "hot spot" in human cancer.

In order to evaluate the role of mtDNA mutations in cervical lesions progression, cervical specimens (from 79 women, 29-65 years old) were investigated. DNA was isolated (High Pure PCR Template, Roche Diagnostics) from cervical cells from patients with different cytology (normal cervical epithelium, ASCUS-Atypical Squamous Cells of Undetermined Significance, LGSIL-Low-Grade Intraepithelial Lesion, HGSIL-High-Grade Intraepithelial Lesion and SCC-Squamous Cell Carcinoma) and tested for HPV DNA presence (Linear Array HPV Genotyping Test, Roche Diagnostics). To elucidate a causative role of mtDNA in cervical lesions, mtDNA mutations were investigated using Mutector mtDNA kit (TrimGen Corporation).

In patients with normal and ASCUS cytology, mtDNA mutations were absent. 16.66% of LGSIL patients presented mutations in D-loop region whereas 28.57% HGSIL cases showed mutations in mtDNA. Mutations were detected in 66.66% cases of SCC cases. These studies provide strong evidence that instability in the D-loop region of mtDNA may be involved in cervical dysplasia. We suggested that mtDNA mutations may play a role in cervical precursor lesions and cancer but their role in the mechanism of carcinogenesis remains to be solved.

Keywords: HPV, cervical cancer, mitochondrial DNA

REFERENCES

1. **Motoyama S, Ladines-llave CA, Villaneueva SL, Maruo T**. The role of human papilloma virus in the molecular biology of cervical carcinogenesis. *Kobe J Med Sci* 2004. **50**: 9-19.

2. **Zur Hausen H**. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002. **2:** 342-350.

3. **Klaes R, Woerner SM, Ridder R, Wentzensen N, Duerst M, Schneider A, Lotz B, Melsheimer P, von Knebel Doeberitz M**. Detection of high risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. *Cancer Res* 1999. **59**: 6132-6139.

4. **Howley PM, Scheffner M, Munger K.** Oncoproteins encoded by the cancer-associated papillomavirus target the products of retinoblastoma and p53 tumor suppressor genes. *Quant Biol* 1991. **56**: 149-155.

5. **Spitokovsky D, Aengeneyndt F, Braspenning J, Doeberitz VK**. p53-independent growth regulation of cervical cancer cells by the papillomavirus E6 oncogene. *Oncogene* 1996. **13**: 1027-1035.

6. **Wallace DC**. Mitochondrial diseases in man and mouse. *Science* 1999. **283**: 1482-1488.

7. **Wallace DC**. A mitochondrial paradigm for degenerative diseases and aging. Ageing vulnerability: causes and interventions. Novartis Found Symp 2001. **235**: 247-263.

8. **Melov S, Shoffner JM, Kaufman S, Wallace DC**. Marked increase in the number and variety of mitochondrial DNA rearrangements in aging human skeletal muscle. *Nucleic Acid Res* 1995. **23**: 4122-4126.

9. **Melov S, Hinerfeld D, Esposito L, Wallace DC**. Multi-organ characterization of mitochondrial genomic rearrangements in ad libitum and caloric restricted mice show striking somatic mitochondrial DNA rearrangements with age. *Nucleic Acids Res* 2007. **25**: 974-982.

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10. **Wallace DC, Murdock DG**. Mitochondria and dystonia: the movement disorder connection. *Proc Natl Acad Sci* USA 2009. **96**: 1817-1819.

11. **Fliss MS, Usadel H, Caballero OL, Wu L, Buta MR, Eleff SM, Jen J, Sidransky D**. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. *Science* 2000. **28**: 2017-2019.

12. **Nomoto S, Yamashita K, Koshikawa K, Nakao A, Sidransky D**. Mitochondrial d-loop mutations as clonal markers in multicentric hepatocellular carcinoma and plasma. *Clin Cancer Res* 2002. **8**: 481-487.

13. **Ha PK, Tong BC, Westra WH, Sanchez-Cespedes M, Parrella P, Zahurak M, Sidransky D, Califano JA**. Mitochondrial c-tract alteration in premalignant lesions of the head and neck: a marker for progression and clonal proliferation. *Clin Cancer Res* 2002. **8**: 2260-2265.

14. **Sanchez-Cespedes M, Parrella P, Nomoto S, Cohen D, Xiao Y, Esteller M, Jernomino C, Jordan RC, Nicol T, Koch WM, Schoenberg M, Mazzarelli P, Fazio VM, Sidransky D**. Identification of a mononucleotide repeat as a major target for mitochondrial DNA alterations in human tumors. *Cancer Res* 2001. **61**: 7015-7019.

15. **Jones JB, Song JJ, Hempen PM, Parmigiani G, Hruban RH, Kern SE**. Detection of mitochondrial DNA mutations in pancreatic cancer offers a "mass"-ive advantage over detection of nuclear DNA mutations. *Cancer Res* 2006. **61**: 299-304.

16. **Penta JS, Johnson RM, Wachsman JT, Copeland WC**. Mitochondrial DNA in human malignancy. *Mutat Res* 2001. 488: 119-133.

17. **Holt I, Harding AE, Morgan-hughes JA**. Deletion of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 1998. 331: 717-719.

18. **Levin BC, Cheng H, Reeder DJ**. A human mitochondrial DNA standard reference material for quality control in forensic identification, medical diagnosis, and mutation detection. *Genomics* 1999. **55**: 135-146.

19. **Alonso A, Martin P, Albarran C, Aquilera B, Garcia O, Guzman A, Oliva H, Sancho M**. Detection of somatic mutations in the mitochondrial DNA control region of colorectal and gastric tumors by heteroduplex and single-strand conformation analysis. *Electrophoresis* 2007. **18**: 682-685.

20. **Suzuki M, Toyooka S, Miyajima K, Iizasa T, Fujisawa T, Bekele NB, Gazdar AF**. Alterations in the mitochondrial displacement loop in lung cancers. *Clin Cancer Res* 2003. **9**: 5636-5641.

21. **Heerdt BG, Chen J, Stewart LR, Augenlicht LH**. Polymorphisms, but lack of mutations or instability, in the promotor region of the mitochondrial genome in human colonic tumors. *Cancer Res* 1994. **54**: 3912-3915.

22. **Liu VW, Shi HH, Cheung AN, Chiu PM, Leung TW, Nagley P, Wong LC, Ngan HY**. High incidence of somatic mitochondrial DNA mutations in human ovarian carcinomas. *Cancer Res*. 2001. **61**: 5998-6001.

23. **Okochi O, Hibi K, Uemura T, Inoue S, Takeda S, Kaneko T, Nakao A**. Detection of mitochondrial DNA alterations in the serum of hepatocellular carcinoma patients. *Clin Cancer Res* 2002. **8**: 2875-2878.

24. **Rosson D, Keshgegian AA**. Frequent mutations in the mitochondrial control region DNA in breast tissue. *Cancer Lett* 2004. **215**: 89-94.

25. **Anton G, Peltecu G, Socolov D, Cornitescu F, Bleotu C, Sgarbura Z, Teleman S, Iliescu D, Botezatu A, Goia CD, Huica I, Anton AC**. Type-specific human papillomavirus detection in cervical smears in Romania. APMIS 2011. **119**: 1-9.

26. **Klaunig JE, Kamendulis LM**. The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol* 2004. **44**: 239-67.

27. **Coller HA, Khrapko K, Bodyak ND, Nekhaeva E, Herrero-Jimenez P, Thilly WG**. High frequency of homoplasmic mitochondrial DNA mutations in human tumors can be explained without selection. *Nat Genet* 2005. **28**: 147-150.

28. **Tamura G, Nishizuka S, Maesawa C, Suzuki Y, Iwaya T, Sakata K, Endoh Y, Motoyama T.** Mutations in mitochondrial control region DNA in gastric tumours of Japanese patients. *Eur J Cancer* 1999. 35: 316-319.

IN VITRO ANTIMICROBIAL ACTIVITY OF ROMANIAN MEDICINAL PLANTS HYDROALCOHOLIC EXTRACTS ON PLANKTONIC AND ADHERED CELLS

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ABSTRACT

The aim of this study was to assess the antibacterial and antifungal potential of some Romanian medicinal plants, arnica - *Arnica montana*, wormwood - *Artemisia absinthium* and nettle - *Urtica dioica*. In order to perform this antimicrobial screening, we obtained the vegetal extracts and we tested them on a series of Gram-positive and Gram-negative bacteria, and also against two fungal strains. The vegetal extracts showed antimicrobial activity preferentially directed against the planktonic fungal and bacterial growth, while the effect against biofilm formation and development was demonstrated only against *S. aureus* and *C. albicans*. Our *in vitro* assays indicate that the studied plant extracts are a significant source of natural alternatives to antimicrobial therapy, thus avoiding antibiotic therapy, the use of which has become excessive in recent years.

Keywords: hydroalcoholic plant extracts, antimicrobial activity, microbial biofilms

REFERENCES

1. **Scarlat Mihai-Alin and Tohaneanu Monica**, Phytotherapy bases, Ed. World Galaxy, 2009.
2. **Ana Garcia-Lafuente, Eva Guillamon, Ana Villares Mauricio, A. Rostagno Jose Alfredo Martinez**. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflamm. Res*. (2009) **58**:537-552.
3. **Ilhami Gulcin, O. Irfan Küfrevioglu, Münir Oktay, Mehmet Emin Büyükokuroglu.** Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.) *Journal of Ethnopharmacology* **90** (2004) 205-215.
4. **Bijan Farzami, D. Ahmadvand, S. Vardasbi, F.J. Majin, Sh. Khaghani**. Induction of insulin secretion by a component of Urtica dioica leave extract in perifused Islets of Langerhans and its in vivo effects in normal and streptozotocin diabetic rats. *Journal of Ethnopharmacology* **89** (2003) 47-53.
5. **Costantine F. Daher, Karmen G. Baroody, George M. Baroody.** Effect of Urtica dioica extract intake upon blood lipid profile in the rats. *Fitoterapia* **77** (2006) 183-188.
6. **Endalkachew Nibret, Michael Wink**, Volatile components of four Ethiopian Artemisia species extracts and their in vitro anti-trypanosomal and cytotoxic activities, *Am. J. Trop. Med. Hyg.,* **70**(2), 2004, pp. 128-132.
7. **Kundan Singh Bora, Anupam Sharmab.** Phytochemical and pharmacological potential of Artemisia absinthium Linn. and Artemisia asiatica. *Journal of Pharmacy Research* 2010, **3**(2),325-328.
8. **James D. Adams Jr, Cecilia Garcia**. Women's Health Among the Chumash. *eCAM* **2006**;3(1)125-131.
9. **Junchi Ueda, Jiro Kato.** Isolation and Identification of a Senescence-promoting Substance from Wormwood *(Artemisia absinthium L.)*. *Plant Physiol*. (1980) **66**, 246-249.
10. **Karin Rath, Katja Taxis, Gitta Walz, Christoph H. Gletier, Shu-Ming Li, Lutz Heide**. Pharmacokinetic Study of Artemisin After Oral Intake of a Traditional Preparation of Artemisia Annua L. (Annual Wormwoood). *Am. J. Trop. Med. Hyg.*, **70**(2), 2004, pp. 128-132.
11. **Lucia Moldovan, Alexandra Gaspar, Liana Toma, Oana Craciunescu, Crina Saviuc.** Comparison of Polyphenolic Content and Antioxidant Capacity of Five Romanian Traditional Medicinal Plants. *Revista de Chimie*, vol. 62/2011 (in press).
12. **Rodney M. Donlan.** 2002. Biofilms: Microbial Life on Surfaces. Emerg Infect Dis., 2002 September; **8**(9): 881-890.
13. **Rauha JP.** 2000. Antimicrobial effects of finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol*., 2000, **56**: 3-12.

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ASSESSING THE EFFICIENCY OF FREE LIGHT CHAIN ASSAY IN MONITORING PATIENTS WITH MULTIPLE MYELOMA BEFORE AND AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION ALONG WITH SERUM PROTEIN ELECTROPHORESIS AND SERUM PROTEIN IMMUNOFIXATION

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ABSTRACT

Monoclonal gammopathies are a group of disorders, referred to as paraproteinaemias, dysproteinaemias or immunoglobulinopathies, associated with monoclonal proliferation of plasma cells. Monoclonal immunoglobulin secreted by these cells is an indicator of clonal proliferation. The aim of this study is to analyze the efficiency of three methods: serum protein electrophoresis (SPE), serum protein immunofixation (IFE) and FLC (free light chain) assay for the diagnosis and monitoring of the tumor burden in multiple myeloma.

In this study we have presented the dynamic evolution of 7 patients with intact immunoglobulin multiple myeloma (IIMM) (2 IgG, κ; 3 IgG, λ; 1 IgA, κ; 1 IgA, λ) and 2 patients with light chain multiple myeloma before and after autologous peripheral blood stem cell transplantation (PBSCT). All 7 patients fulfilled the four criteria for the diagnosis of IIMM: bone marrow plasma cells exceeding 20%, lytic bone lesions, identification and quantification of M protein by scanning densitometry of electrophoresis gels, IFE (immunofixation protein electrophoresis) confirmed and typed the M protein. All patients had been given cytotoxic chemotherapy (VAD or VELCADE) before autologous (PBSCT).

In two of the patients with IIMM both SPE and κ/λ ratio fell towards normal range after autologous PBSC and both reported a relapse of the disease after 23 months and 19 months respectively. SPE could not normalize after chemotherapy and transplantation in three patients with IIMM, the κ/λ ratio being the only marker used to monitor the tumor kill. In one patient the κ/λ ratio could not normalize even after PBSCT still indicating the presence of plasma cell disorder at the time when IFE was still negative. 16 months after PBSCT both SPE and FLC indicated a relapse of the disease.

Classical SPE failed to demonstrate the presence of M- protein in light chain multiple myeloma, the diagnosis being established by using IFE and the FLC assay. Because IFE is a qualitative method and its interpretation may be sometimes subjective, FLC was the only method used to follow the disease course.

The measurement of κ/λ ratio proved to be more sensitive than SPE, IFE and the levels of free light chains κ or λ individually indicating whether the treatment is effective or not.

Keywords: multiple myeloma, autologous stem cell transplantation, free light chain, immunofixation

REFERENCES

1. **Kyle Robert A. *et al.*** Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. *Br J Haematol*, 2003. **121**(5): 749-57.
2. **Katzmann JA, Kyle RA, Joanne Benson, Larson DR., Melissa R Snyder, Lust John A, Rajkumar SV, Angela Dispenzieri**. Screening Panels for Detection of Monoclonal Gammopathies. *Clin Chem*, 2009. **55**: 1517-1522.
3. **Angela Dispenzieri, Zhang L, Katzmann JA, Melissa Snyder, Emily Blood, Roberta DeGoey, Kimberly Henderson, Kyle RA, Oken MM, Bradwell AR, Greipp PR.** Appraisal of immunoglobulin free light chain as a marker of response. *Blood*, 2008. **111**: 4908-4915.
4. **Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell AR, Kyle RA**. Serum reference intervals and diagnostic ranges for free κ and free λ immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin Chem*, 2002. **48**: 1437-44.

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5. **Dispenzieri Angela, Kyle R, Merlini G, Miguel JS, Ludwig H, Hajek R, et al**. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia*, 2009. **23**: 215-24.
6. **Kyle R A and Rajkumar S V.** Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. *Leukemia*, 2009. 23: 3-9.
7. **Katzmann JA, Stankowski-Drengler TJ, Kyle RA, Lockington Karen S, Snyder Melissa R, Lust JA, Angela Dispenzieri.** Specificity of serum and urine protein electrophoresis for the diagnosis of monoclonal gammopathies. *Clin Chem*, 2010. **56**: 1899-1900.
8. **Xavier Bossuyt, Ann Bogaerts, Gilberte Schiettekatte, Norbert Blanckaert.** Serum protein electrophoresis and immunofixation by a semiautomated electrophoresis system. *Clin Chem*, 1998. **44**: 944-949.
9. **Katzmann JA, Abraham RS, Angela Dispenzieri, Lust JA, Kyle RA.** Diagnostic Performance of Quantitative and Free Light Chain Assays in Clinical Practice. *Clin Chem*, 2005. **51**: 878-881.
10. **Bradwell AR**. Serum free light chain measurements move to center stage. *Clin Chem,* 2005. **51**: 805-807.
11. **Keren DF, Alexanian R, Goeken JA, Gorevic PD, Kyle RA, Tomar RH**. Guidelines for clinical and laboratory evaluation of patients with monoclonal gammopathies. *Arch Pathol Lab Med*, 1999. **123**: 106-7.
12. **Durie BG, Harousseau JL, Miguel JS, Blade J, Barlogie B, Anderson K, et al.** International uniform response criteria for multiple myeloma. *Leukemia*, 2006. **20**: 1467-1473
13. **Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, Showell PJ, Drayson MT, Drew R.** Highly sensitive automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem*, 2001. **47**: 673-80.
14. **Bradwell AR, Carr-Smith HD, Mead GP, Drayson MT.** Serum free light chain immunoassays and their clinical application. *Clinical and Applied Immunology Reviews*, 2002. **3**: 17-33.
15. **Mead G.P., Carr-Smith, H.D., Drayson, M.T., Morgan, G.J., Child,J.A., Bradwell,A.R.** Serum free light chains for monitoring multiple myeloma. *Br. J. Haematology*, 2004. **126**: 348-354.
16. **Kyrtsonis MC, Vassilakopoulos TP, Kafasi N, Sachanas S, Papadogiannis A, et al.** Prognostic value of serum free light chain ratio at diagnosis in multiple myeloma. *Br J Haematol*, 2007. **137**: 240-243.
17. **Tate JR, Devinder Gill, Cobcroft Ralph, Hickman Peter E.** Practical considerations for the measurement of free light chains in serum. *Clin Chem,* 2003. **49:** 1252-1257.

IMMUNODETECTION OF ADDED GLYCOMACROPEPTIDE IN MILK FORMULAS AND MILK POWDERS

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ABSTRACT

The present study aimed the detection of fraudulent manipulation of milk powder with a low cost component - whey powder, by applying the immunochromatographic assay to identify glycomacropeptide. Five commercial milk powder samples of various brands from the national market were analyzed: lactose enriched milk powder type 26, two whole milk powders, vitamin enriched milk powder and full cream milk powder. Our results showed additional whey (1-2%) in 60% of the selected samples after casein removal by precipitation with 20% trichloracetic acid. Another investigated sample - the enriched UHT milk for children aged 4-12 years - proved addition of whey. Other two commercial toddler formula milk powder samples of different brands were used for comparison for the presence of glycomacropeptide. The first sample which was regularly labeled as containing whey protein concentrate was found positive for glycomacropeptide in accordance with the label information, while the second one not containing whey proteins as specified by the product label, was found negative for glycomacropeptide, these two samples being in accordance with the actual legislation.

Keywords: whey proteins, k-casein glycomacropeptide, milk formula, immunochromatography.

REFERENCES

1. **Jensen RG.** Handbook of milk composition, Academic Press, San Diego, 1995.
2. **Saito T, Yamaji A, Itoh T.** A new isolation method of caseinoglycopeptide from sweet cheese whey. *J Dairy Sci* 1991. **74**: 2831-2837.
3. **Brody EP.** Biological activities of bovine glycomacropeptide, *British J Nut* 2000. **84**: S39-S46.
4. **Eigel MN, Buttler JE, Ernstorm CA, Farrell HM, Harwalker VR, Jenness R, Whitney RM.** Nomenclature of proteins of cow's milk: Fifth revision. *J Dairy Sci* 1984. **67**: 1599-1631.
5. ***Official Journal of the EU L-142, Regulation no 1898-87 of the Conseil of 2 July 1987.
6. **Lechner E, Klostermeyer H.** Nachweis einer Verfälschung von Magermilchpulver mit Molkenpulver (polarographische Methode). *Milchwissenschaft* 1981. **36**: 267-270.
7. **Meisel H.** Application of fourth derivative spectroscopy to quantitation of whey protein and casein in total milk protein. *Milchwissenschaft* 1990. **50**: 247-251.
8. **Miralles B, Bartolomé B, Amigo L, Ramos M.** Comparison of three methods to determine the whey protein to total protein ratio in milk. *J Dairy Sci* 2000. **83**: 2759-2765.
9. **Meisel H, Carstens J.** Vergleichende Untersuchungen zur Bestimmung des Molkenprotein- und Caseinanteils in Milchprodukten mittels Casein-Phosphor-Methode, SDS-Elektrophorese und Polarographie. *Milchwissenschaft* 1989. **44**: 271-277.
10. **Recio I, Olieman C.** Determination of denatured serum proteins in the casein fraction of heat-treated milk by capillary zone electrophoresis. *Electrophoresis* 1996. **17**: 1228-1233.
11. **Bordin G, Cordeiro RF, de la Calle B, Rodriguez AR.** Identification and quantification of major bovine milk proteins by liquid chromatography. *J Chromatogr A* 2001. **928**: 63-76.
12. **Greiner SP, Kellen GJ, Carpenter DE.** A rapid immunoturbidimetric method for whey proteins in nonfat dry milk and buttermilk. *J Food Sci* 1985. **50**(4): 1106-1109.
13. **De Souza EMT, Arruda SF, Brandao PO, Siqueira EM de Almeida.** Electrophoretic analysis to detect and quantify additional whey in milk and dairy beverages. *Ciênc Tecnol Aliment* 2000. **20** (3): 314-317.

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14. **Ferreira MPLVO, Oliveira MBPP.** Determination of caseinomacropeptide by an RP-HPLC method and monitoring of the addition of rennet whey to powdered milk. *J Liq Chrom Relat Tech* 2003. **26**: 99-107.
15. **Molle D, Leonil J.** Quantitative determination of bovine *k*-casein macropeptide in dairy products by Liquid chromatography/Electrospray coupled to mass spectrometry (LC-ESI/MS) and Liquid chromatography/Electrospray coupled to tandem mass spectrometry (LC-ESI/MS/MS). *Int Dairy J* 2005. **15**: 419-428.
16. **Recio I, Garcia-Risco MR, Lopez-Fandino R, Olano A, Ramos M.** Detection of rennet whey solids in UHT milk by capillary electrophoresis. *Int Dairy J* 2000. **10**: 333-338.
17. **Reid JR, Coolbear T, Ayers JS, Coolbear KP**. The action of chymosin on k-casein and its macropeptide: effect of pH and analysis of products of secondary hydrolysis. *Int Dairy J* 1998. **7**: 559-569.
18. **Bitri L, Rolland MP, Besançon P.** Immunological detection of bovine caseinomacropeptide in ovine and caprine dairy products. *Milchwissenschaft* 1993. **48**: 367-371.
19. **Calvo MM.** Influence of fat, heat treatments and species on milk rennet clotting properties and glycomacropeptide formation. *European Food Research and Technology* 2002. **214**:182-185.
20. **Kelleher SL, Chatterton D, Nielsen K, Lönnerdal B**. Glycomacropeptide and α-lactalbumin supplementation of infant formula affects growth and nutritional status in infant rhesus monkeys. *Am J Clin Nutr* 2003. **77**:1261-1268.
21. **Sharma SK, Hill AR, Mittal GS**. An improved method to measure glycomacropeptides (GMP) in renneted milk. *Milchwissenschaft* 1993. **48**:71-73.
22. **Recio I, Lopez-Fandino R, Olano A, Olieman C, Ramos M.** Study of the formation of caseinomacropeptides in stored UHT milk by capillary electrophoresis. *J Agric Food Chem* 1996. **44**:3955-3959.
23. **Miralles B, Bartolome B, Ramos M, Amigo L.** Determination of whey protein to total protein ratio in UHT milk using fourth derivative spectroscopy. *Int Dairy J* 2000. **10**:191-197.
24. **Wong PYY, Nakamura S, Kitts DD**. Functional and biological activities of casein glycomacropeptide as influenced by lipophilization with medium and long chain fatty acid. *Food Chemistry* 2006. **97**:310-317.

BACTERIAL EXTRACT CANTASTIM ACTIVATES MACROPHAGES VIA TLR-2

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ABSTRACT

CANTASTIM is a second generation bacterial immunomodulator. The aim of this study was to examine the mechanism by which bacterial immunomodulator CANTASTIM induces production of inflammatory cytokines in monocytes/macrophages. Proinflammatory cytokines were induced in PMA-differentiated THP-1 cells by stimulation with TLR agonists and CANTASTIM in the presence or absence of anti-TLR blocking antibodies or isotype matched control antibodies. Also, RNA interference was used to knockdown TLR2 or TLR4 expression in PMA-differentiated THP-1 cells before stimulation. As expected, induction of TNF-α and IL-6 by TLR4 agonist LPS was inhibited in a significant manner by anti-TLR4 but not by anti-TLR2 antibody. Unexpectedly, treatment with anti-TLR2 blocking antibody inhibited only IL-6 production induced by Pam3CSK4 while the level of TNF-alpha was unchanged. When cells were stimulated by TLR2 agonist heat-killed *Listeria monocytogenes* the release of TNF-α was significantly attenuated by anti-TLR2 antibodies. Silencing of TLR2 led to a statistically significant inhibition of TNF-α secretion induced by TLR2 agonist while siRNA silencing of TLR4 did not affect the response to TLR2 agonist. Cells exposed to CANTASTIM produced significant levels of pro-inflammatory cytokines but the levels were lower than LPS-stimulated cells. Production of both cytokines was inhibited by treatment with anti-TLR2 blocking antibody and not by anti-TLR4 antibody. Silencing of TLR2 led to a statistically significant inhibition of TNF-α secretion induced by CANTASTIM while silencing of TLR4 had no effect on the response to CANTASTIM. These results support the hypothesis that CANTASTIM may exert its immunomodulatory and adjuvant activities through interaction of its bacterial components with TLR2.

Keywords: TLR, macrophages, CANTASTIM

REFERENCES

1. **Sălăgeanu A, Ceacăreanu B, Popescu D, Istrate N, and Szegli G.** Experimental studies on bacterial product CANTASTIM derived from Pseudomonas aeruginosa. II. Protective effect in *Salmonella typhimurium* infection. *Roum Arch Microbiol Immunol* 1997. **56**(1): 17-26.
2. **Sălăgeanu A and Szegli G.** Experimental studies on the bacterial product CANTASTIM derived from *Pseudomonas aeruginosa*. V. Protective effect in endotoxin shock. *Roum Arch Microbiol Immunol* 1999. **58**(2): 101-109.
3. **Caraş I, Serbănescu IF, Grigorescu A, and Sălăgeanu A.** Experimental studies on bacterial product cantastim derived from Pseudomonas aeruginosa. VII. Activation of immune cells of healthy controls and cancer patients. *Roum Arch Microbiol Immunol* 2005. **64**(1): 5-10.
4. **Lerescu L, Ţucureanu C, Caraş I, and Sălăgeanu A.** Cytokine profiling by multiplex immunoassay as an effective approach to assess immunomodulatory activity of bacterial product CANTASTIM. *Roum Arch Microbiol Immunol* 2006. **65**(1): 53-58.
5. **Anghelache IF, Caraş I, and Sălăgeanu A.** Adjuvant properties of bacterial product cantastim. *Roum Arch Microbiol Immunol* 2007. **66**(1): 17-21.
6. **Caraş I, Ţucureanu C, Lerescu L, and Sălăgeanu A.** Bacterial product cantastim derived from *Pseudomonas aeruginosa* induces migration and maturation of dendritic cells. *Roum Arch Microbiol Immunol* 2007. **66**(1): 5-9.
7. **Medzhitov R.** Recognition of microorganisms and activation of the immune response. *Nature* 2007. **449** (7164): 819-826.
8. **Kawai T and Akira S.** TLR signaling. *Semin Immunol* 2007. **19**(1): 24-32.
9. **Kulkarni R, Behboudi S, and Sharif S.** Insights into the role of Toll-like receptors in modulation of T cell responses. *Cell Tissue Res* 2011. **343**(1):141-152.
10. **Zanoni I and Granucci F.** Regulation of antigen uptake,

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migration, and lifespan of dendritic cell by Toll-like receptors. *J Mol Med* 2010. **88**(9): 873-880.
11. **Masihi KN.** Fighting infection using immunomodulatory agents. *Expert Opin Biol Ther* 2001.**1**(4): 641-653.
12. **Braido F, Tarantini F, Ghiglione V, Melioli G, and Canonica GW.** Bacterial lysate in the prevention of acute exacerbation of COPD and in respiratory recurrent infections. *Int J Chron Obstruct Pulmon Dis* 2007. **2**(3): 335-345.
13. **Nikolova M, Stankulova D, Taskov H, Nenkov P, Maximov V and Petrunov B.** Polybacterial immunomodulator Respivax restores the inductive function of innate immunity in patients with recurrent respiratory infections. *Int Immunopharmacol* 2009. **9**(4): 425-432.
14. **Uehori J, Fukase K, Akazawa T, Uematsu S, Akira S, Funami K, Shingai M, Matsumoto M, Azuma I, Toyoshima K, Kusumoto S, Seya T.** Dendritic cell maturation induced by muramyl dipeptide (MDP) derivatives: monoacylated MDP confers TLR2/TLR4 activation. *J Immunol* 2005. **174** (11): 7096-7103.
15. **Uehori J, Matsumoto M, Tsuji S, Akazawa T, Takeuchi O, Akira S, Kawata T, Azuma I, Toyoshima K, Seya T.** Simultaneous blocking of human Toll-like receptors 2 and 4 suppresses myeloid dendritic cell activation induced by Mycobacterium bovis bacillus Calmette-Guerin peptidoglycan. *Infect Immun* 2003. **71**(8): 4238-4249.
16. **Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F, Liu YJ.** Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* 2001. **194**(6): 863-869.
17. **Peiser M, Koeck J, Kirschning CJ, Wittig B, and Wanner R.** Human Langerhans cells selectively activated via Toll-like receptor 2 agonists acquire migratory and CD4+ T cell stimulatory capacity. *J Leukoc Biol* 2008. **83**(5): 1118-1127.
18. **Stoitzner P, Zanella M, Ortner U, Lukas M, Tagwerker A, Janke K, Lutz MB, Schuler G, Echtenacher B, Ryffel B, Koch F, Romani N.** Migration of langerhans cells and dermal dendritic cells in skin organ cultures: augmentation by TNF-alpha and IL-1beta. *J Leukoc Biol* 1999. **66**(3): 462-470.
19. **Taillardet M, Haffar G, Mondière P, Asensio MJ, Pléau-Pison T, Burdin N, Defrance T, Genestier L.** Toll-Like Receptor Agonists Allow Generation of Long-Lasting Antipneumococcal Humoral Immunity in Response to a Plain Polysaccharidic Vaccine. *J Infect Dis* 2010. **202**(3): 470-479.
20. **Guan Y, Omueti-Ayoade K, Mutha SK, Hergenrother PJ, and Tapping RI.** Identification of Novel Synthetic Toll-like Receptor 2 Agonists by High Throughput Screening. *J Biol Chem* 2010.**285**(31): 23755-23762.
21. **Imanishi T, Hara H, Suzuki S, Suzuki N, Akira S, and Saito T.** Cutting edge: TLR2 directly triggers Th1 effector functions. *J Immunol* 2007. **178**(11): 6715- 6719.
22. **Querec T, Bennouna S, Alkan S, Laouar Y, Gorden K, Flavell R, Akira S, Ahmed R, Pulendran B.** Yellow fever vaccine YF-17D activates multiple dendritic cell subsets via TLR2, 7, 8, and 9 to stimulate polyvalent immunity. *J Exp Med* 2006. **203**(2): 413-424.
23. **Liu H, Komai-Koma M, Xu D, and Liew FY.** Toll-like receptor 2 signaling modulates the functions of CD4+ CD25+ regulatory T cells. *Proc Natl Acad Sci* U S A 2006. **103**(18):7048-7053.
24. **Liu X, Wetzler LM, and Massari P.** The PorB porin from commensal Neisseria lactamica induces Th1 and Th2 immune responses to ovalbumin in mice and is a potential immune adjuvant. *Vaccine* 2008, **26**(6): 786-796.
25. **Sieling PA, Chung W, Duong BT, Godowski PJ, and Modlin RL.** Toll-like receptor 2 ligands as adjuvants for human Th1 responses. *J Immunol* 2003. **170**(1): 194-200.
26. **Martinez J, Huang X and Yang Y.** Direct TLR2 Signaling Is Critical for NK Cell Activation and Function in Response to Vaccinia Viral Infection. *PLoS Pathog* 2010. **6**(3): e1000811.
27. **Wenink MH, Santegoets KC, Broen JC, van Bon L, Abdollahi-Roodsaz S, Popa C, Huijbens R, Remijn T, Lubberts E, van Riel PL, van den Berg WB, Radstake TR.** TLR2 promotes Th2/Th17 responses via TLR4 and TLR7/8 by abrogating the type I IFN amplification loop. *J Immunol* 2009. **183**(11): 6960-6970.

IMPLANT MICROPARTICLES - A NEW CONCEPT FOR NON-INVASIVE CANCER THERAPY

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ABSTRACT

Some progress in cancer research was possible in recent years mainly due to important advances in nanotechnology. However, clinical use of nanomaterials is still hindered by limitations. In search of better performance and control of inoculated materials, the efficiency and toxicity of SBBC implant particles was assessed. B16 tumoral cells (murine melanoma) were subjected to SBCC particles using *in vitro* and *in vivo* experimental models. *In vitro* experiments concerning the growth inhibition of tumoral cells using SBCC particles were performed by Flow Cytometry and by MTT Assay. *In vivo* experimental model (C57BL/6 mice) was used to complete this investigation: weight, viability and tumoral dimension were monitored. An anti-proliferative activity on B16 tumoral cells and an ability to produce apoptosis were observed. A reduction of tumoral volume and a 54% survival rate in the treated animals compared to the controls was obtained. Our preliminary results showed that the SBCC implants were effective against B16 melanoma cells, while there is no toxicity associated.

Keywords: tumor, anti-proliferative, Flow Cytometry, SBCC implant particles

REFERENCES

1. **Ciofani G, Raffa V, Menciassi A, Cuschieri A.** Boron nitride nanotubes: an innovative tool for nanomedicine. *Nano Today* 2009. **4**: 8-10.
2. **Zhao W, Karp JM.** Tumour targeting: Nanoantennas heat up. *Nat Mater* 2009. **8**: 453-454.
3. **Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R.** Nanocarriers: Emerging Platforms for Cancer Therapy. *Nat Nanotech* 2007. **2**:751-760.
4. **Mukherjee P, Bhattacharya R, Wang P, Wang L, Basu S, Nagy JA, Atala A, Mukhopadhyay D, Soker S.** Antiangiogenic properties of gold nanoparticles. *Clin Cancer Res* 2005. **11**:3530-3534.
5. **Galal SA, Hegab KH, Kassab AS, Rodriguez ML, Kerwin MS, El-Khamry AA, El Diwani HI.** New transition metal ion complexes with benzimidazole-5-carboxylic acid hydrazides with antitumor activity. *European Journal of Medicinal Chemistry* 2009. **44:** 1500-1508.
6. **Gao J, Xu B.** Applications of Nanomaterials Inside Cells. *Nano Today* 2009. **4**: 37-51.
7. Johan **PE Karlberg, Marjorie A Speers.** Reviewing Clinical Trials: A Guide for the Ethics Committee 2010. Publisher: Karlberg, Johan Petter Einar ISBN 978-988-19041-1-9
8. **Kim KY.** Nanotechnology platforms and physiological challenges for cancer therapeutics. *Nanomed Nanotechnol Biol Med* 2007. **3**: 103-110.
9. **Surdu-Bob CC, Badulescu M.** Patent pending. Deposit No. A/00754/2009 - Romania.
10. **Mosmann T.** Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods* 1983. **65**(1-2): 55-63.

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