The Expression of Toll-like Receptors 2, 4, 5, 7 and 9 in Merkel Cell Carcinoma

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Abstract. *Aim: We sought to clarify whether the expression of toll-like receptors (TLR) in Merkel cell carcinoma (MCC) is linked to tumor and patient characteristics, especially the presence of Merkel cell polyoma virus (MCV). Materials and Methods: The study comprised of 128 patients with data on Merkel cell polyomavirus (MCV) status and clinical features were included in the study. Immunohistochemistry for TLR expression was performed on tissue microarray (TMA) slides. Results: TLR 2, 4, 5, 7 and 9 expression was noted in most of the tumor specimens. Decreased expression of TLR 9 correlated strongly with MCV positivity. Cytoplasmic TLR 2 expression correlated with small tumor size, while nuclear TLR 2 and TLR 5 expressions with larger tumors. Increased nuclear TLR 4 expression and decreased TLR 7 expression were associated with older age. Conclusion: TLR 2, 4, 5, 7 and 9 appear to reflect certain clinicopathological variables and prognostic markers of MCC tumors.*

Merkel cell carcinoma (MCC), a primary neuroendocrine skin cancer is considered one of the most aggressive cutaneous malignancies (1). It occurs typically in fair-skinned elderly individuals and more frequently than expected in immunosuppressed individuals. The course of disease correlates inversely with large tumour size, metastasis-positive nodal status and distant metastasis (2).

In 2008, a previously unknown double-stranded DNA polyomavirus was found in MCC tumours and metastases and named Merkel cell polyoma virus (MCV) (3), which is clonally integrated in MCC tumor genomes. MCV is, hence, considered to be an important causative agent for the development of a significant proportion of MCCs (4, 5) and it has been suggested that possibly all MCCs harbour MCV (6). According to the "hit and run" hypothesis, MCV is a causal factor for MCCs. Once the succeeding carcinogenic mutations have occurred, the infected cells begin to lose detectable viral antigen, while the oncogenic phenotype is retained (7). None of these hypotheses has, however, gained popularity and the current perspective favours the interpretation that there are both MCV-positive and -negative tumours in MCC.

Infectious agents have been recognized as causative factors in 20% of cancers (8). The impaired immunological functions of the host, such as an immunosuppression or immunocompromise, precedes the occurrence of certain malignancies of which a vast majority are indeed virus-induced cancers (9). Reducing the viral burden through elimination of the virus-infected cells is an important way to control these cancers and immunocompetent hosts are at a higher risk of developing a virus-induced cancer (10).

Toll-like receptors (TLRs) are key players in innate immune recognition. In healthy tissues, TLRs support homeostasis by detecting harmful molecular structures of both pathogens (11-13) and injured or dead cells (14, 15). Activation of TLRs initiates intracellular signalling pathways leading to gene transcription regulation, cell proliferation, differentiation, mitosis, cell-cycle regulation, apoptosis and secretion of co-stimulatory factors and cytokines (16, 17). The same signaling pathways are also involved in certain abnormal conditions. In dysplasia and cancer, the activation of TLRs can launch cascades leading to malignant transformation by inducing, for instance, apoptosis resistance and immune escape of cancerous cells by producing such immune modulatory cytokines as nitric oxide synthase 2, cyclooxygenase 2 (COX-2), vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β) (18-20). On the other hand, TLRs in

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Key Words: Merkel cell carcinoma-prognosis-Merkel cell polyoma virus-infection.

Table I. *The demographic data and tumour-related factors of the Merkel cell carcinoma patients.*

All	128
Gender	
Male	39 (31.0 %)
Female	87 (69.0 %)
Age, years	
≤50	3 (2.4 %)
51-69	28 (22.2 %)
≥70	95 (75.4 %)
Tumor location	
Head and neck	69 (54.8 %)
Lower extremity	25 (19.8 %)
Upper extremity	23 (18.3 %)
Trunk	9 (7.1 %)
Tumor size	
Mean diameter, mm	19.2
≤20mm	88 (69.8 %)
>20mm	38 (30.2 %)
Merkel cell polyoma virus status	
Positive	83 (65.9 %)
Negative	30 (23.8 %)
Not evaluated	13 (10.3 %)
American Joint Committee on Cancer status at presentation	
I	81 (64.3 %)
II	30 (23.8 %)
III	10 (7.9 %)
IV	5 (4.0 %)
Overall survival	
3-year	49.6 %
5-year	36.0 %
Merkel cell carcinoma specific survival	
3-year	75.7 %
5-year	68.8 %

Table II. *Expression of Toll-like receptors (TLRs) 2, 4, 5, 7 and 9 stratified by their scores.*

	Number of cases				Missing
Immuno-expression	0 (%)	1 (%)	2 (%)	3 (%)	values
TLR 2 (cp)	17 (22.1)	25 (32.5)	18 (23.4)	17 (22.1)	49
TLR 2 (n)	42 (54.5)	5 (6.5)	13 (16.9)	17 (22.1)	49
TLR 4 (cp)	19 (25.3)	24 (32.0)	24 (32.0)	8 (10.7)	51
TLR 4 (n)	64 (85.3)	6 (8.0)	3 (4.0)	2 (2.7)	51
TLR 5	69 (82.1)	7 (8.3)	4 (4.8)	4 (4.8)	42
TLR 7	NA	18 (23.4)	30 (39.0)	29 (37.7)	49
TLR 9 (cp)	1 (1.4)	18 (25.4)	30 (42.3)	22 (31.0)	55

n, Nuclear staining; cp, cytoplasmic staining; NA, non-available.

cancer can also cause tumour inhibition, thus, the description of the role of TLRs in cancer is relatively ambiguous (21-23).

The aim of the present study was to further explore the markers of inflammation in MCC by evaluating immunohistochemical expression of Toll-like receptors 2, 4, 5, 7 and 9 in multi-tissue array samples and study if their expression is linked to MCV, tumour characteristics and disease outcome parameters.

Materials and Methods

The study protocol was approved by the Ethics Committee of Helsinki University Central Hospital. The Ministry of Health and Social Affairs granted permission to collect patient data and the National Authority for Medicolegal Affairs to collect and analyse the tissue samples.

Data on patients diagnosed with MCC in Finland during 1979-2004 were obtained from the Finnish Cancer Registry and the files of Helsinki University Hospital. Detailed data on patient and tumour characteristics, including tumour size, location, local recurrence, local and systemic metastasis, as well as survival, were obtained from the hospital and primary health care centre files. The clinical characteristics of the patients are illustrated in Table I.

The formalin-fixed, paraffin-embedded tissue blocks of patients included in this survey were retrieved from the archives of the Department of Pathology. The histology and immunohistochemical verification of the tumour samples has been described previously in detail (24). For histological diagnosis of MCC, we required that morphology was compatible with MCC in light microscopy, positivity for CK-20 and negative staining for TTF-1. The longest diameter of the tumour was measured from hematoxylin and eosin stained slides. For this current survey, we utilized a multi-tissue array (TMA), including specimens from the 128 patients that had been recruited to our previous study on the clinical features of MCC and information on MCV status. The TMA block included an average of two 0.7 mm specimens from each tumour.

Patients were retrospectively staged according the American Joint Committee on Cancer (AJCC) staging system (2). In brief, patients with primary MCC with no clinical or pathological evidence of regional or distant metastases were divided into two stages: stage I for primary tumours ≤ 20 mm and stage II for primary tumours >20 mm. Stages I and II were further divided into substages A and B according to a method of nodal evaluation; for stage A, microscopic evaluation of draining lymph nodes was performed, while stage B was defined as those evaluated clinically. For patients with histologically proven micrometastasis, the stage was defined as IIIA; this included all tumour sizes. Stage IV comprised tumours of any size and regional lymph node status with systemic spread of metastasis.

Immunohistochemistry. Four-μm sections were cut from the TMA blocks. Slides were deparaffinised in xylene and rehydrated through an alcohol gradient to water. For antigen retrieval, slides were heated in 98°C Tris-HCl buffer (pH 8.5) for 20 min in PT-module (LabVision UK Ltd, Newmarket, UK). Immunohistochemical staining was performed with Autostainer 480 (LabVision UK Ltd) with Dako REAL Envision Detection System, Peroxidase/ DAB+, Rabbit/Mouse (Dako, Glostrup, Denmark) as described (25). The following primary antibodies were used: polyclonal rabbit anti-human TLR 2, 4 (1:50, sc-10739 and sc-10741) and 9 (1:100, sc-25468; Santa Cruz Biotechnology, Santa Cruz, CA USA) and monoclonal rabbit anti-human TLR 7 (1:300, IMG-581A; Imgenex, San Diego, CA, USA).

Interpretation of staining. Immunohistochemical staining was evaluated semi-quantitatively; percentages of positive cells were estimated and scored. Scores ranged from 0 (no staining), 1 (mild staining, 30% or less), 2 (moderate staining, 31-70%), to 3 (strong staining, more than 71%). For TLR-2 and TLR 4, both cytoplasmic and nuclear positivity and intensity was scored, whereas for TLR-9 the cytoplasm intensity was scored. For TLR 5 and TLR 7, the stratification for nuclear or cytoplasmic staining was omitted and immunoexpression was scored as such.

Statistical analysis. The SPSS Version 20.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis of the data. The Mann-Whitney U test was used for comparing the staining patterns with MCV-status. The Kendall tau-b was used for comparing the staining patterns with primary tumour diameter. The Spearman rank correlation was used to compare the staining patterns with age. The log-rank test was used in univariate analysis. The level of statistical significance was established below 0.05.

Results

The staining results of TLRs 2, 4, 5, 7 and 9 in MCC are summarized in Table II.

Correlations between TLRs and MCV status. The expression of TLR 9 varied according to MCV status. Increased positivity of TLR 9, with a mean score of 2.47, was connected with MCV negativity, while lower TLR 9 expression, mean score 1.87, was associated with MCV positivity (p=0.012). Expression scores of TLRs other than 9 did not correlate with MCV status.

TLR expression and tumour diameter. When comparing the TLR expressions with tumour size, cytoplasmic TLR 2 demonstrated strong expression in small tumours; the mean expression in tumours with diameter ≤ 10mm was 2.00 compared to 1.31 of larger tumours (p=0.022), (Figure 1). In contrast, larger than 10mm tumours showed higher nuclear TLR 2 expression of 1.23, compared to mean nuclear expression of 0.44 in smaller tumors (p=0.025). TLR 5 stained mostly mildly in tumors >10mm, with a mean expression of 0.40, compared with negative TLR 5 expression in ≤10mm tumors (p=0.040). No correlation was noticed between the tumour diameter and other TLRs immunoexpression.

TLR expression and other clinicopathological parameters. Tumors showed no distinct patterns in MCV status, recurrences or metastases according to TLR 2 expression, even when we analysed two categories according to their size (≤10 mm and >10 mm). Despite the fact that TLR 2 expression decreased in larger tumours, no prognostic value for TLR 2 expression or tumour size was established. Similarly, the expression of other TLRs did not prognosticate survival significantly.

Expression of nuclear TLR 4 was increased (p=0.005) (Figure 2) and TLR 7 (Figure 3) was decreased (p=0.025) in elderly MCC patients. No correlation between TLR expression and AJCC stage, primary tumor localization or disease recurrence was noticed.

Discussion

We analyzed the expression of five TLRs, namely TLR 2, 4, 5, 7 and 9 immunohistochemically in MCC. The expression of the studied TLRs had significant correlations with previously validated prognostic factors, such as MCV status, tumor size and patient age. Decreased expression of TLR 9 connected strongly with MCV positivity. Other significant results in this present study was high cytoplasmic TLR 2 expression in small tumours and enhanced nuclear TLR 2 and TLR 5 expression in larger tumors. Older age of the MCC patients associated with increased nuclear TLR 4 expression and decreased TLR 7 expression.

We and others have shown that MCV infection is an essential causal factor to MCC and that MCV positive and negative tumours differ from each other by means of molecular and clinical features (5, 26, 27). Other oncogenic viruses, such as Epstein-Barr virus, papillomavirus 16 and hepatitis B virus, down-regulate TLR 9 expression (28-31). Similarly in MCC, MCV-transfected cells down-regulate TLR 9 *via* the transcription factor C/EBPβ, a mechanism distinct from other oncogenic viruses (32). In the present current study, we found evidence that TLR 9 expression is decreased in MCV-positive MCCs, although we failed to find prognostic implication for TLR 9 in MCC. The exact biological and functional role of TLR 9 in MCC remains pending.

The course of disease in MCC is characterized by rapid tumour growth. The tumour may grow into considerable size in just a few months, although the median time from lesion appearance to biopsy is only about three months (33). Apart from being an important feature in staging, tumour size (especially tumor size >2 cm) remains an important prognostic factor (34). Studies on assessing the effect of tumour thickness on outcome have, however, failed to report dependence (35, 36). For instance, our previous study shows that small tumor size at presentation is not a marker for indolent tumour pathobiology (37).

TLR 2 is involved in the recognition of a wide range of pathogen-associated molecular patterns (PAMPs) derived from bacteria, fungi, parasites and viruses (38). Therefore, our current finding, that the expression of TLR 2 is not connected with MCV status but correlated with tumor size, may apply to other oncogenic factors apart from MCV. In colorectal carcinoma, the invasiveness and ability to migrate is mediated through a TLR 2-dependent pathway, whereas inhibition of TLR 2 signalling reduces malignant potential of cancer cells (39). In hepatocellular carcinoma, the ligation

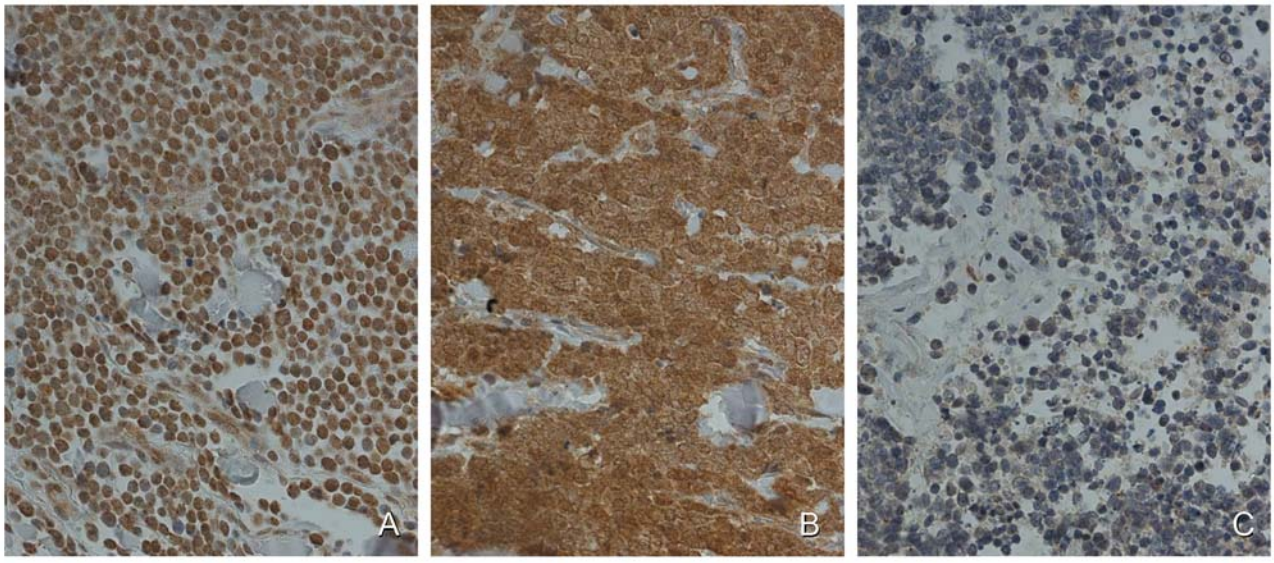

Figure 1. *TLR-2 expression in MCC. (A) Strong nuclear expression, (B) Strong cytoplasmic expression, (C) negative TLR sample.*

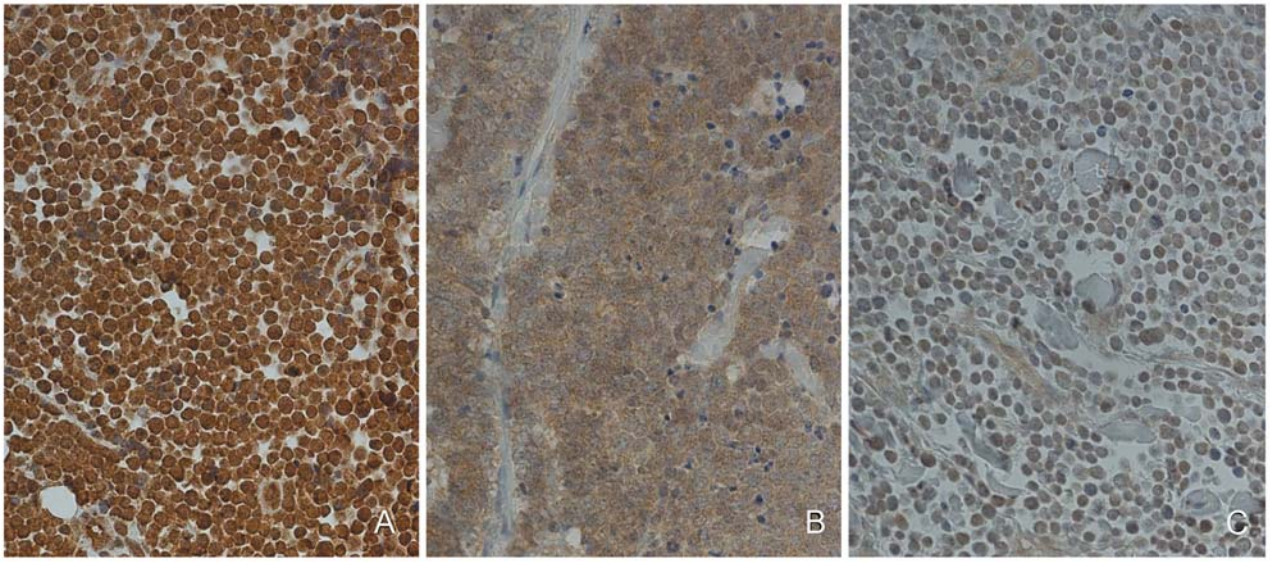

Figure 2. *TLR-4 expression in MCC.(A) Strong nuclear, (B) Mild cytoplasmic, (C) negative for TLR 4 sample.*

of TLR 2 increases tumour growth (40) and in a gastric cancer mouse model, the activation of TLR 2 prolongs tumour cell survival and proliferation rather than tumour-related inflammation (41). These results obtained from other types of malignancies are in line with our current ones; large MCC tumors lack cytoplasmic TLR 2 expression, even though the nuclear expression was increased. Based on our results, we may suggest that the switch of cytoplasmic TLR 2 expression to nuclear positivity is associated with tumour growth in MCC regardless of MCV status. TLR 5, which was also connected with larger tumour diameter in this study, increases carcinogenesis in a variety of different cancer types, such as oral, tongue, oesophageal and cervical cancers (42-44). The role of TLR 5 in cancer is, however, controversial, as the activation of TLR 5 promotes tumour migration and invasion in salivary gland adenocarcinoma, (45) but also mediates the immune system's antitumor activity in breast cancer (46).

Many studies have suggested a pivotal role for TLR 4 in cancer. For example, in follicular thyroid cancer, a strong TLR 4 expression predicts poor outcome (47) and in cutaneous malignant melanoma, strong TLR 4 expression reveals an

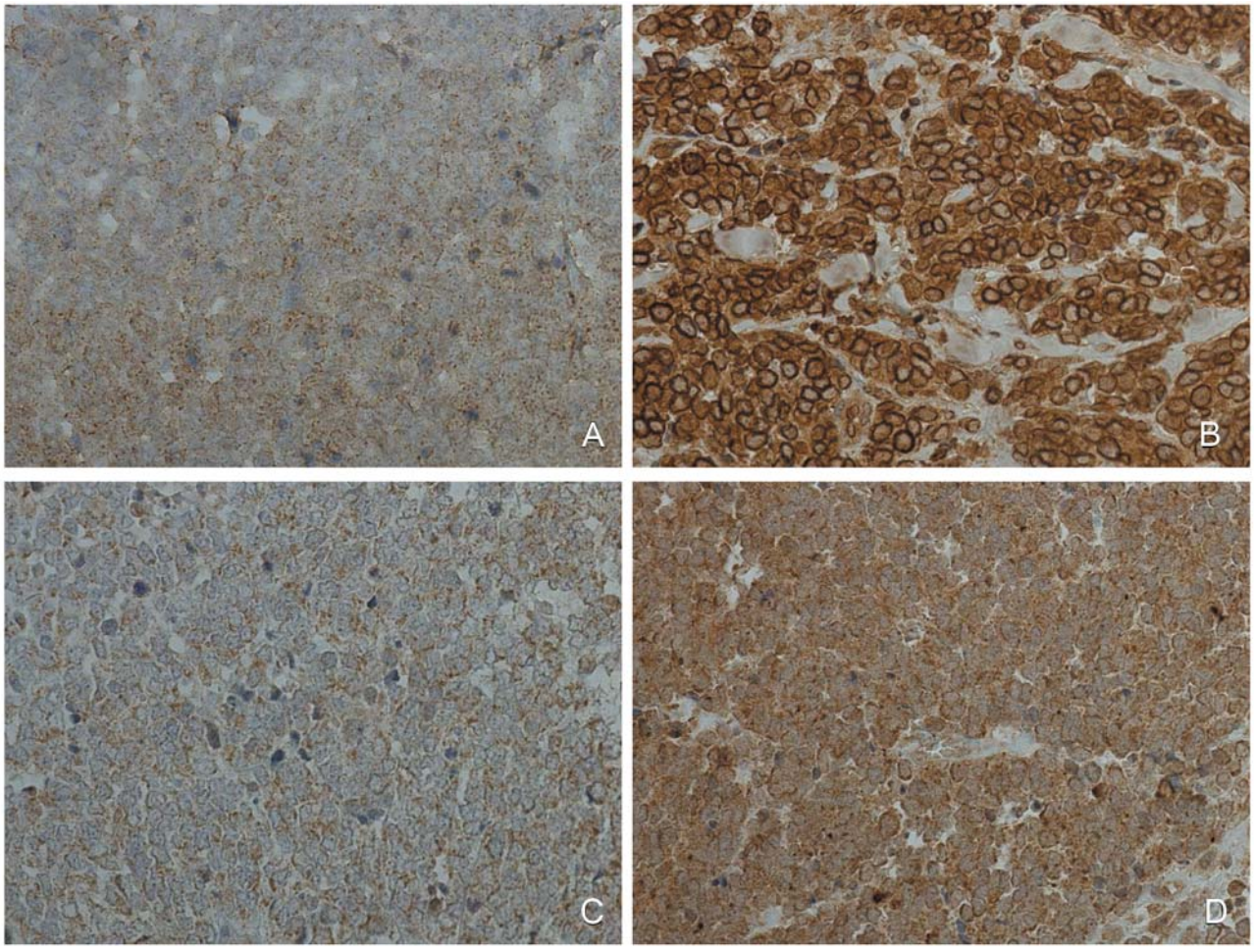

Figure 3. *TLR-7 and-9 expression in MCC. Mild cytoplasmic TLR7 expression (A) and strong expression (B). Mild cytoplasmic TLR-9 expression (C) and strong expression (D).*

increased risk for recurrences (48). Concerning MCC, we found a higher TLR 4 expression in tumors from elderly patients. Despite its unclear role in cancer, treating cancer through the TLR 4 pathway is considered to be a promising alternative. Currently, the feasibility of a TLR 4 agonist, the Glucopyranosyl Lipid Adjuvant-Stable Emulsion (GLA-SE) is studied (http://clinicaltrials.gov/ show/NCT02035657). The desired anti-cancer activity of this agonist is mediated through dendritic cells, which are manipulated to produce T helper-1 cell-promoting cytokines, tumour necrosis factor-α, interleukin (IL)-1β, IL-6 and IL-12 in a manner mimicking the actions induced by pathogens (49). Like TLR 4, the activation of TLR 7 in cancer may also have both beneficial and harmful consequences as the agonist imiquimod activates necrosis and apoptosis in oral cancer, whereas, in lung cancer, TLR 7 activation leads to increased chemoresistance and cancer cell survival (50, 51). We failed, however, to find any connection between outcome or tumor characteristics and TLR 4 and 7 expression in MCC, whilst they were connected with older age. Peripheral blood leukocytes and dendritic cells show an age-related decrease in TLR 7 expression (52, 53). TLR 4 expression is up-regulated in muscle tissue of elderly people but down-regulated in peripheral blood leukocytes and colon mucosa cells. On the basis of age-related TLR expression pattern variation, we could speculate that our finding of TLR 4 and 7 expression patterns in elderly people might reflect the overall impact of aging.

In conclusion, we report that the expression of TLRs was connected with previously established clinical and epidemiological prognostic markers and provides molecular evidence to strengthen their importance. The methodology used in this current study, measuring the immunohistochemical expression of TLRs, should raise new questions on the pathobiological basis for these prognostic markers. In general, we could suggest that TLRs do have a role in MCC tumor biology.

Conflicts of Interest

Each Author declares no financial conflicts of interest with regard to the data presented in this manuscript.

Acknowledgements

The Authors would like to thank Päivi Peltokangas and Elina Aspiala for excellent technical assistance. This study received financial support from EVO Research Funds from Specified Government Transfer and the Sigrid Jusélius Foundation Medicinska understödsföreningen Liv och Hälsa.

References

1 Becker JC: Merkel cell carcinoma. Ann Oncol 21 *Suppl 7*: vii81-vii85, 2010.
2 Edge SB, Byrd DR, Compton CC and (eds.): *Merkel cell carcinoma*. New York, NY: Springer, 2010.
3 Feng H, Shuda M, Chang Y and Moore PS: Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science *319*: 1096-1100, 2008.
4 Bouvard V, Baan RA, Grosse Y, Lauby-Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N and Straif K: Carcinogenicity of malaria and of some polyomaviruses. Lancet Oncol *13*: 339-340, 2012.
5 Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T and Joensuu H: Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. J Natl Cancer Inst *101*: 938-945, 2009.
6 Rodig SJ, Cheng J, Wardzala J, DoRosario A, Scanlon JJ, Laga AC, Martinez-Fernandez A, Barletta JA, Bellizzi AM, Sadasivam S, Holloway DT, Cooper DJ, Kupper TS, Wang LC and DeCaprio JA: Improved detection suggests all Merkel cell carcinomas harbor Merkel polyomavirus. J Clin Invest 122: 4645-4653, 2012.
7 Houben R, Schrama D, Alb M, Pfohler C, Trefzer U, Ugurel S and Becker JC: Comparable expression and phosphorylation of the retinoblastoma protein in Merkel cell polyoma virus-positive and negative Merkel cell carcinoma. Int J Cancer *126*: 796-798, 2010.
8 Parkin DM: The global health burden of infection-associated cancers in the year 2002. Int J Cancer *118*: 3030-3044, 2006.
9 Grulich AE, van Leeuwen MT, Falster MO and Vajdic CM: Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet *370*: 59-67, 2007.
10 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144*: 646-674, 2011.
11 Janeway CA, Jr.: Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harbor symposia on quantitative biology *54 Pt 1*: 1-13, 1989.
12 Medzhitov R and Janeway CA, Jr.: Innate immunity: the virtues of a nonclonal system of recognition. Cell *91*: 295-298, 1997.
13 Kumar H, Kawai T and Akira S: Toll-like receptors and innate immunity. Biochemical and biophysical research communications *388*: 621-625, 2009.
14 Matzinger P: Tolerance, danger, and the extended family. Annual review of immunology *12*: 991-1045, 1994.
15 Matzinger P: The danger model: a renewed sense of self. Science *296*: 301-305, 2002.
16 Basith S, Manavalan B, Yoo TH, Kim SG and Choi S: Roles of toll-like receptors in cancer: a double-edged sword for defense and offense. Archives of pharmacal research 35: 1297-1316, 2012.
17 Kawai T and Akira S: Pathogen recognition with Toll-like receptors. Current opinion in immunology *17*: 338-344, 2005.
18 He W, Liu Q, Wang L, Chen W, Li N and Cao X: TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. Molecular immunology *44*: 2850-2859, 2007.
19 Huang B, Zhao J, Li H, He KL, Chen Y, Chen SH, Mayer L, Unkeless JC and Xiong H: Toll-like receptors on tumor cells facilitate evasion of immune surveillance. Cancer Res *65*: 5009-5014, 2005.
20 Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, Hsu D, Xu R, Harpaz N, Dannenberg AJ, Subbaramaiah K, Cooper HS, Itzkowitz SH and Abreu MT: Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. Gastroenterology *133*: 1869-1881, 2007.
21 Lowe EL, Crother TR, Rabizadeh S, Hu B, Wang H, Chen S, Shimada K, Wong MH, Michelsen KS and Arditi M: Toll-like receptor 2 signaling protects mice from tumor development in a mouse model of colitis-induced cancer. PLoS One *5*: e13027, 2010.
22 Matijevic T, Marjanovic M and Pavelic J: Functionally active toll-like receptor 3 on human primary and metastatic cancer cells. Scandinavian journal of immunology *70*: 18-24, 2009.
23 Bauer AK, Dixon D, DeGraff LM, Cho HY, Walker CR, Malkinson AM and Kleeberger SR: Toll-like receptor 4 in butylated hydroxytoluene-induced mouse pulmonary inflammation and tumorigenesis. J Natl Cancer Inst *97*: 1778-1781, 2005.
24 Sahi H, Koljonen V, Kavola H, Haglund C, Tukiainen E, Sihto H and Bohling T: Bcl-2 expression indicates better prognosis of Merkel cell carcinoma regardless of the presence of Merkel cell polyomavirus. Virchows Arch *461*: 553-559, 2012.
25 Ahmed Haji Omar A, Haglund C, Virolainen S, Hayry V, Atula T, Kontio R, Rihtniemi J, Pihakari A, Salo T, Hagstrom J and Sorsa T: Epithelial and stromal syndecan-1 and -2 are distinctly expressed in oral- and cutaneous squamous cell carcinomas. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology *42*: 389-395, 2013.
26 Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T and Joensuu H: Merkel cell polyomavirus infection, large T antigen, retinoblastoma protein and outcome in Merkel cell carcinoma. Clin Cancer Res *17*: 4806-4813, 2011.
27 Sahi H, Savola S, Sihto H, Koljonen V, Bohling T and Knuutila S: RB1 gene in Merkel cell carcinoma: hypermethylation in all tumors and concurrent heterozygous deletions in the polyomavirus-negative subgroup. APMIS *122*: 1157-1166, 2014.
28 Fathallah I, Parroche P, Gruffat H, Zannetti C, Johansson H, Yue J, Manet E, Tommasino M, Sylla BS and Hasan UA: EBV latent membrane protein 1 is a negative regulator of TLR9. Journal of immunology *185*: 6439-6447, 2010.
29 van Gent M, Griffin BD, Berkhoff EG, van Leeuwen D, Boer IG, Buisson M, Hartgers FC, Burmeister WP, Wiertz EJ and Ressing ME: EBV lytic-phase protein BGLF5 contributes to TLR9 downregulation during productive infection. Journal of immunology *186*: 1694-1702, 2011.

30 Hasan UA, Bates E, Takeshita F, Biliato A, Accardi R, Bouvard V, Mansour M, Vincent I, Gissmann L, Iftner T, Sideri M, Stubenrauch F and Tommasino M: TLR9 expression and function is abolished by the cervical cancer-associated human papillomavirus type 16. Journal of immunology *178*: 3186-3197, 2007.
31 Vincent IE, Zannetti C, Lucifora J, Norder H, Protzer U, Hainaut P, Zoulim F, Tommasino M, Trepo C, Hasan U and Chemin I: Hepatitis B virus impairs TLR9 expression and function in plasmacytoid dendritic cells. PLoS One *6*: e26315, 2011.
32 Shahzad N, Shuda M, Gheit T, Kwun HJ, Cornet I, Saidj D, Zannetti C, Hasan U, Chang Y, Moore PS, Accardi R and Tommasino M: The T antigen locus of Merkel cell polyomavirus downregulates human Toll-like receptor 9 expression. J Virol *87*: 13009-13019, 2013.
33 Heath M, Jaimes N, Lemos B, Mostaghimi A, Wang LC, Penas PF and Nghiem P: Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. J Am Acad Dermatol *58*: 375-381, 2008.
34 Koljonen VS: Merkel cell carcinoma. World J Surg Oncol *4*: 7, 2006.
35 Andea A, Coit DC and Busam K: An analysis of morphologic parameters as prognostic markers in merkel cell carcinoma. Am J Dermatopathol *28*: 228, 2006.
36 Goldberg SR, Neifeld JP and Frable WJ: Prognostic value of tumor thickness in patients with Merkel cell carcinoma. J Surg Oncol *95*: 618-622, 2007.
37 Kukko HM, Koljonen VS, Tukiainen EJ, Haglund CH and Bohling TO: Vascular invasion is an early event in pathogenesis of Merkel cell carcinoma. Mod Pathol *23*: 1151-1156, 2010.
38 Akira S, Uematsu S and Takeuchi O: Pathogen recognition and innate immunity. Cell *124*: 783-801, 2006.
39 Guo H, Chen Y, Hu X, Qian G, Ge S and Zhang J: The regulation of toll-like receptor 2 by miR-143 suppresses the invasion and migration of a subset of human colorectal carcinoma cells. Mol Cancer *12*: 10.1186, 2013.
40 Huang B, Zhao J, Shen S, Li H, He KL, Shen GX, Mayer L, Unkeless J, Li D, Yuan Y, Zhang GM, Xiong H and Feng ZH: Listeria monocytogenes promotes tumor growth via tumor cell toll-like receptor 2 signaling. Cancer Res *67*: 4346-4352, 2007.
41 Tye H, Kennedy CL, Najdovska M, McLeod L, McCormack W, Hughes N, Dev A, Sievert W, Ooi CH, Ishikawa TO, Oshima H, Bhathal PS, Parker AE, Oshima M, Tan P and Jenkins BJ: STAT3-driven upregulation of TLR2 promotes gastric tumorigenesis independent of tumor inflammation. Cancer cell *22*: 466-478, 2012.
42 Kauppila J, Mattila A, Karttunen T and Salo T: Toll-like receptor 5 (TLR5) expression is a novel predictive marker for recurrence and survival in squamous cell carcinoma of the tongue. British journal of cancer *108*: 638-643, 2013.
43 Helminen O, Huhta H, Takala H, Lehenkari PP, Saarnio J, Kauppila JH and Karttunen TJ: Increased Toll-like receptor 5 expression indicates esophageal columnar dysplasia. Virchows Arch *464*: 11-18, 2014.
44 Kim WY, Lee JW, Choi JJ, Choi CH, Kim TJ, Kim BG, Song SY and Bae DS: Increased expression of Toll-like receptor 5 during progression of cervical neoplasia. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society *18*: 300-305, 2008.
45 Park JH, Yoon HE, Kim DJ, Kim SA, Ahn SG and Yoon JH: Toll-like receptor 5 activation promotes migration and invasion of salivary gland adenocarcinoma. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology *40*: 187-193, 2011.
46 Cai Z, Sanchez A, Shi Z, Zhang T, Liu M and Zhang D: Activation of Toll-like receptor 5 on breast cancer cells by flagellin suppresses cell proliferation and tumor growth. Cancer Res *71*: 2466-2475, 2011.
47 Hagstrom J, Heikkila A, Siironen P, Louhimo J, Heiskanen I, Maenpaa H, Arola J and Haglund C: TLR-4 expression and decrease in chronic inflammation: indicators of aggressive follicular thyroid carcinoma. Journal of clinical pathology *65*: 333-338, 2012.
48 Eiro N, Ovies C, Fernandez-Garcia B, Alvarez-Cuesta CC, Gonzalez L, Gonzalez LO and Vizoso FJ: Expression of TLR3, 4, 7 and 9 in cutaneous malignant melanoma: relationship with clinicopathological characteristics and prognosis. Archives of dermatological research *305*: 59-67, 2013.
49 Schreibelt G, Tel J, Sliepen KH, Benitez-Ribas D, Figdor CG, Adema GJ and de Vries IJ: Toll-like receptor expression and function in human dendritic cell subsets: implications for dendritic cell-based anti-cancer immunotherapy. Cancer immunology, immunotherapy: CII *59*: 1573-1582, 2010.
50 Ahn MY, Kwon SM, Cheong HH, Park JH, Lee J, Min SK, Ahn SG and Yoon JH: Toll-like receptor 7 agonist, imiquimod, inhibits oral squamous carcinoma cells through apoptosis and necrosis. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology *41*: 540-546, 2012.
51 Cherfils-Vicini J, Platonova S, Gillard M, Laurans L, Validire P, Caliandro R, Magdeleinat P, Mami-Chouaib F, Dieu-Nosjean MC, Fridman WH, Damotte D, Sautes-Fridman C and Cremer I: Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. J Clin Invest *120*: 1285-1297, 2010.
52 Rosenstiel P, Derer S, Till A, Hasler R, Eberstein H, Bewig B, Nikolaus S, Nebel A and Schreiber S: Systematic expression profiling of innate immune genes defines a complex pattern of immunosenescence in peripheral and intestinal leukocytes. Genes and immunity *9*: 103-114, 2008.
53 Panda A, Qian F, Mohanty S, van Duin D, Newman FK, Zhang L, Chen S, Towle V, Belshe RB, Fikrig E, Allore HG, Montgomery RR and Shaw AC: Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response. Journal of immunology *184*: 2518-2527, 2010.

Received December 7, 2014
Revised December 18, 2014
Accepted December 23, 2014