Metabolic reprogramming and angiogenesis in primary cutaneous Merkel cell carcinoma: expression of hypoxia-inducible factor-1α and its central downstream factors

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Abstract

Background Metabolic reprogramming and altered gene expression mediated by hypoxia-inducible factors play crucial roles during tumour growth and progression. Nevertheless, studies analysing the expression of hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor-A (referred to as VEGF throughout the manuscript), VEGF receptor-2 (VEGFR-2), VEGF receptor-3 (VEGFR-3), glucose transporter-1 (Glut-1), monocarboxylate transporter 4 (MCT4) and carbonic anhydrase IX (CAIX) in primary cutaneous MCC are lacking but are warranted to shed more light on MCC pathogenesis and to potentially provide new therapeutic options.

Objectives To analyse the immunohistochemical expression of hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor-A (VEGF-A), VEGF receptor-2 (VEGFR-2), VEGF receptor-3 (VEGFR-3), glucose transporter-1 (Glut-1), monocarboxylate transporter 4 (MCT4) and carbonic anhydrase IX (CAIX) in primary cutaneous MCC.

Methods The 16 paraffin-embedded primary cutaneous MCCs (Merkel cell polyomavirus (McPyV) positive/negative: 11/5) were analysed by immunohistochemistry, namely HIF-1α, VEGF, VEGFR-2 (KDR), VEGFR-3 (FLT4), Glut-1, MCT4 and CAIX. An established quantification score (QS) was applied to quantitate the protein expression by considering the percentage of positive tumour cells (0: 0%; 1: up to 1%; 2: 2–10%; 3: 11–50%; 4: >50%) in relation to the staining intensity (0: negative; 1: low; 2: medium; 3: strong).

Results HIF-1α was expressed in all MCCs and predominantly found at the invading edges of tumour margins. The HIF-1α downstream factors Glut-1, MCT4 and CAIX were expressed in 13 of 16 MCC (81%), 14 of 16 MCC (88%) and 16 of 16 MCC (100%), respectively. Interestingly, VEGF and VEGFR-2 were not expressed in tumour cells, whereas VEGFR-3 was expressed in all MCCs. HIF-1α was expressed significantly stronger in McPyV+ tumours (QS: 10.36 ± 2.41) than in McPyV− tumours (QS: 5.40 ± 1.34; P = 0.002). Similarly, VEGFR-3 was also expressed significantly stronger in McPyV+ tumours (QS: 10.00 ± 2.52) than in McPyV− tumours (QS: 5.40 ± 3.43, P = 0.019).

Conclusions Our data provide first evidence for a role of HIF-1α in induced metabolic reprogramming contributing to MCC pathogenesis. The metabolic signatures of McPyV+ and McPyV− tumours seem to show relevant differences.

Conflict of Interest None declared.

Funding sources None.

Introduction

Merkel cell carcinoma (MCC) is an uncommon primary cutaneous neuroendocrine cancer that typically affects sun-damaged skin in elderly and/or immunocompromised patients. The aggressive behaviour of MCC is reflected by its high rates of local recurrence and lymph node metastasis, and low 5-year survival rates (51% for local disease and 14% for distant disease). Approximately 80% of MCC cases are positive for the Merkel cell polyomavirus (McPyV) that was reported by Feng et al. in 2008. McPyV+ tumours show better prognosis than their McPyV− counterparts. However, the pathogenesis of MCC is not completely understood despite recent advances in
immunology and cancer genetics, and studies focusing on MCC-related metabolic profiles and angiogenesis are rare.

Hypoxia resulting from excessive cancer cell proliferation distant from an oxygen-providing vasculature plays a crucial role in carcinogenesis. Decreased oxygen availability leads to metabolic reprogramming and altered gene expression, which enables tumour adaptation to a hypoxic environment. Hypoxia-inducible factor-1α (HIF-1α) is an oxygen-sensitive transcription factor that mediates the transcription of over 200 target genes that orchestrate tumour proliferation and progression under hypoxia. Under normoxic conditions, HIF-1α is targeted for proteasomal degradation via a multistep process including hydroxylation and polyubiquitination. In contrast, hypoxia leads to HIF-1α stabilization and its translocation into the nucleus where it dimerizes with obligate partners and binds to so-called hypoxia-response elements within the promoter regions of numerous target genes. Expression of HIF-1α-targeted genes including Glut-1, MCT4, CAIX and VEGF subsequently initiates various processes like metabolic switching (glycolysis instead of oxidative phosphorylation), angiogenesis and cell proliferation, all of which stimulate carcinogenesis and metastatic spread. HIF-1α overexpression has been reported in a variety of solid tumours and their metastases and is associated with a poor prognosis (e.g. in melanoma or oral squamous cell carcinoma) and tumour invasiveness. Upregulation of glucose transporter-1 (Glut-1), the most common human glucose transporter, contributes to an enhanced glucose metabolism in rapidly proliferating tumour cells. Several studies have examined Glut-1 expression in melanocytic nevi and melanoma as well as in non-melanoma skin cancer. However, there is only one immunohistochemical study on record that has analysed Glut-1 expression in MCC.

In the context of altered metabolism in tumour cells, monocarboxylate transporters (MCTs, especially MCT1 and MCT4) contribute substantially to the hyperglycolytic and acid-resistant phenotype of cancer cells by mediating cellular lactate and proton efflux. MCTs are upregulated in many tumours, and recent evidence highlights that their expression could be used as a biomarker to predict responses to chemotherapy as MCTs are involved in the uptake of chemotherapeutic agents in cancer cells. Remarkably, immunohistochemical studies focussing on MCT4 expression in MCC are lacking. The HIF-1α induced pH regulator, carbonic anhydrase IX (CAIX), also contributes to metabolic reprogramming in cancer cells and has been associated with metastatic spread and poor prognosis in various human tumours including malignant melanoma. However, CAIX has not yet been immunohistochemically analysed in MCC.

Vascular endothelial growth factor (VEGF), a central downstream factor of HIF-1α, is crucial for physiologic and pathologic (lymph-) angiogenesis, and represents a potential target of anticancer therapy. VEGF upregulation has been described in various murine and human cutaneous tumours, indicating a critical role of VEGF in angiogenesis and tumour development in skin cancer. Only few studies have analysed the expression of VEGF and its receptors in MCC.

To the best of our knowledge, this is the first immunohistochemical study analysing the expression of HIF-1α and several of its downstream targets that are involved in metabolic reprogramming and angiogenesis in MCC.

Materials and methods

Specimens

In total, 16 paraffin-embedded primary cutaneous Merkel cell carcinomas (McPyV+/McPyV−: 11/5) were analysed by immunohistochemistry. Additional clinical and histological data are summarized in Table 1.

Immunohistochemistry

Details regarding each antibody, clone, source, dilution and pretreatment are summarized in Table 2. To evaluate the expression of all investigated proteins in the MCC specimens, an established quantification score (QS) was calculated by multiplying the relative proportion of positive tumour cells (levels of positivity: 0: 0%; 1: up to 1%; 2: 2–10%; 3: 11–50%; and 4: >50%) with the value of the staining intensity (level of intensity: 0: negative; 1: low; 2: medium; and 3: strong). Single products were added to obtain a summed score, if multiple intensity ranks were observed in one specimen. Additionally, the expression of epidermal VEGF was evaluated by measuring the staining intensity (level of intensity: 0: negative; 1: low; 2: medium; and 3: strong) of VEGF in epidermal keratinocytes. ‘Blind’ analysis was performed on the specimens by two independent dermatopathologists (FT and WH) without knowledge of the McPyV status, section number or any other clinical data. All cases in which different scores were calculated were discussed together in order to define a uniform score.

Statistical methods

Statistical analysis was performed using the SPSS statistical package (v24.0, SPSS Inc., Chicago, Illinois, USA). Group differences in the expression of the different proteins were tested using the Statistical methods

Table 1 Clinical and histological data

| Feature                  | McPyV+/McPyV− | McPyV/−McPyV+ |
|--------------------------|---------------|---------------|
| Age                      | 81.18 ± 8.32 years | 79.6 ± 4.27 years |
| Sex                      | Female/male   | 6/5           | 1/4          |
| Anatomical side          | Head/trunk/ arm/leg | 1/2/3/5       | 3/1/0/1     |
| Ulceration               | Yes versus no | 1:10          | 2:3          |
| McPyV, Merkel cell polyomavirus | SD, standard deviation | | |
and McPyV was analysed by crosstabs and Fisher’s exact test. Dichotomous variables, sex and ulceration, and McPyV status (age, sex) and histological (ulceration) data. The association of the different protein expression scores and the clinical correlation coefficients were calculated to explore the correlation with medians, interquartile ranges and ranges. Spearman’s rank was considered statistically significant.

Results

Clinical and histological data

The mean (±standard deviation (SD)) age of patients with McPyV+ MCC (female/male: 6/5) was 81.18 (±8.31) years (range: 68–91 years), and the mean (±SD) age of patients with McPyV− MCC (female/male: 1/4) was 79.6 (±4.27) years (range: 74–85 years).

When using the Mann–Whitney U test, the mean age showed no statistically significant difference between McPyV+ tumours and McPyV− tumours (P = 0.766). Furthermore, sex as well as the presence or absence of ulceration was not significantly associated with McPyV status (both P > 0.214). The patient demographics and histologic data are summarized in detail in Table 1.

HIF-1α expression

HIF-1α was expressed in all McPyV+ and McPyV− MCC. HIF-1α expression (mean ± SD) by the measured QS in McPyV+ tumours (10.36 ± 2.41) was significantly stronger than that in McPyV− tumours (5.40 ± 1.34; P = 0.002). The expression of HIF-1α was higher at the invading edges of tumour margins in both subgroups, whereas the central parts of the tumours tended to show weak or no HIF-1α expression (Fig. 1a-c).

VEGF expression

Tumoral VEGF expression was not evident in any of the analysed MCC samples, whereas epidermal VEGF expression was found in all tumours and rated as strong (QS: 3) in all samples. Interestingly, the epidermis directly above the tumour tended to show higher VEGF expression than the epidermis located at the periphery of the MCC (Fig. 1d).

Statistical correlation analyses

Protein expression levels Spearman’s rank correlation upon comparing all MCC cases without McPyV status subgroup stratification revealed that HIF-1α expression was positively correlated with VEGFR-3 expression (correlation coefficient: 0.509,

Table 2 Primary antibodies used for immunostaining

| Antibody | Clone | Source | Company | Dilution | Antigen Retrieval |
|----------|-------|--------|---------|----------|-------------------|
| VEGF     | EP1176Y | Rabbit | Zytomed systems, Berlin, Germany | 1:200 | pH 9.0 |
| VEGFR-2(KDR) | 55B11 | Rabbit | Cell Signaling, Frankfurt, Germany | 1:500 | pH 9.0 |
| VEGFR-3 (FLT4) | KL9 | Mouse | Leica, Nußloch, Germany | 1:10 | pH 9.0 |
| HIF-1alpha | Polyclonal | Rabbit | Bio-Techne, Minneapolis, USA | 1:50 | pH 9.0 |
| Glut-1 | SPM498 | Mouse | Zytomed systems, Berlin, Germany | 1:40 | pH 6.1 |
| MCT4 | D-1 | Mouse | Santa Cruz Biotechnology, Heidelberg, Germany | 1:100 | pH 9.0 |
| CAIX | Polyclonal | Rabbit | Abcam, Cambridge, USA | 1:200 | pH 6.1 |

We detected no VEGFR-2 expression on a protein level in the tumour cells of the investigated MCC samples.

VEGFR-3 expression was evident in all MCC samples irrespective of the McPyV status. VEGFR-3 expression (mean ± SD) was significantly stronger in McPyV+ tumours (10.00 ± 2.52) than in McPyV− MCCs (5.40 ± 3.43, P = 0.019). Figure 1e shows a case of MCC with strong VEGFR-3 expression.

Glut-1 expression

Glut-1 expression was present in 10 of 11 McPyV+ MCC (91%) and in 3 of 5 McPyV− MCC (60%). Glut-1 expression (mean ± SD) in McPyV+ tumours (5.18 ± 2.75) did not differ significantly from that in McPyV− tumours (3.6 ± 3.28; P = 0.432). Figure 1f and g depict a case of MCC with strong Glut-1 expression. Glut-1 expression was positively correlated with the HIF-1α expression in McPyV− tumours (correlation coefficient: 0.913, P = 0.030).

MCT4 expression

MCT4 was expressed in 10 of 11 McPyV+ MCC samples (91%) and in 4 of 5 McPyV− MCC samples (80%). MCT4 expression (mean ± SD) in McPyV+ tumours (5.00 ± 3.06) did not differ significantly from that in McPyV− tumours (4.40 ± 3.36; P = 0.81). A case of MCC with strong expression of MCT4 is shown in Figure 1h and i.

CAIX expression

CAIX expression was observed in all MCC samples irrespective of the McPyV status. CAIX expression (mean ± SD) in McPyV+ tumours (12.18 ± 2.96) did not differ significantly from that in McPyV− tumours (9.40 ± 2.60; P = 0.134). Figure 1j and k depict a case of MCC with strong CAIX expression (Fig. 2).
Figure 1 Expression of HIF-1α and its downstream factors in primary cutaneous Merkel cell carcinoma. (a) Sample showing a Merkel cell carcinoma with strong HIF-1α expression predominantly at the invading tumour margins (HIF-1α staining, original magnification × 25). (b) Close up of the same section depicted in a (original magnification × 200). (c) Central areas of the tumour depicted in a/b lacking HIF-1α expression (original magnification × 200). (d) Section showing a Merkel cell carcinoma with strong epidermal VEGF expression but no tumoral VEGF expression (VEGF staining, original magnification × 25). (e) Sample of a Merkel cell carcinoma with strong VEGFR-3 expression (VEGFR-3 staining, original magnification × 25). (f) Merkel cell carcinoma sample showing Glut-1 expression. (Glut-1 staining, original magnification × 50). (g) Close up of the same tumour depicted in f. Arrows: Erythrocytes serve as positive control for Glut-1 expression. (Glut-1 staining, original magnification × 200). (h) Sample revealing Merkel cell carcinoma with strong MCT4 expression in peripheral tumour areas (MCT4 staining, original magnification × 25). (i) Close up of h (MCT4 staining, original magnification × 200). (j) Merkel cell carcinoma revealing stronger CAIX expression in the invading tumour margins than in the central parts of the tumour (CAIX staining, original magnification × 25). (k) Close up of j (CAIX staining, original magnification × 100).
McPyV showed that within subgroups the correlation was not significant (McPyV: correlation coefficient: 0.160, \( P = 0.638 \), McPyV\(^{-}\): correlation coefficient: 0.703, \( P = 0.185 \)). Furthermore, in McPyV\(^{-}\) tumours, the HIF-1\(\alpha\) expression was positively correlated with the Glut-1 expression (correlation coefficient: 0.913, \( P = 0.030 \)).

The correlation coefficients of the different protein expression scores are summarized in Table 3.

**Protein expression levels and clinical data** Upon analysing the tumours without subgroup stratification, none of the proteins showed expression levels that were significantly associated with patient age, sex or ulceration. However, Glut-1 expression was negatively correlated with sex in the McPyV\(^{+}\) tumours (correlation coefficient: \(-0.825\), \( P = 0.002 \)) due to different mean Glut-1 expression levels (\(\pm 0.00 \)) in male 7.4 (\(\pm 1.52 \)) and female patients 3.33 (\(\pm 2.07 \)) (\( P = 0.009 \)).

**Discussion**

In 2019, the Nobel Prize in physiology or medicine was awarded to the three physician-scientists, Drs. William G. Kaelin, Jr., Peter Ratcliffe and Gregg Semenza, for their pioneering work revealing how cells sense and adapt to oxygen availability.\(^{26}\) HIF-1\(\alpha\), that was first described by Semenza and coworkers in 1995,\(^{27}\) is activated in response to low oxygen levels and is considered as key regulator of oxygen homeostasis as it mediates the transcription of over 200 target genes playing crucial roles during tumour proliferation and progression under hypoxia.\(^{3,4}\)

Here, we analysed the expression of HIF-1\(\alpha\) and a number of its central downstream targets namely Glut-1, MCT4 and CAIX, as well as VEGF, VEGFR-2 and VEGFR-3 in order to elucidate metabolic reprogramming and angiogenesis in MCC.

We found HIF-1\(\alpha\) expression in all of the investigated samples irrespective of the McPyV status. These results indicate that HIF-1\(\alpha\) probably contributes to the pathogenesis of MCC and are in accordance with the findings of other groups that have analysed HIF-1\(\alpha\) expression in cutaneous tumours such as melanoma, basal cell carcinoma and cutaneous squamous cell carcinoma.\(^{5,10}\) Interestingly, upon subgroup analysis, we found a significantly positive correlation of the HIF-1\(\alpha\) expression with the Glut-1 expression only in McPyV\(^{-}\) tumours. These findings indicate differences in the metabolic signatures of McPyV\(^{+}\) and McPyV\(^{-}\) tumours. Nevertheless, due to the relatively small sample number, these findings need to be interpreted cautiously and have to be confirmed in studies including more samples.

Zhong et al. investigated HIF-1\(\alpha\) expression in a number of human cancer types and described the density of HIF-1\(\alpha\) positive cells to be highest at the invading edges of the tumour margins and adjacent to necrotic or strongly vascularized regions.\(^{7}\) Our findings are in accordance with these data, as we also predominantly found HIF-1\(\alpha\) positivity in the invading edges of tumour margins.

Inhibition of HIF-1\(\alpha\) reflects a promising anticancer therapy.\(^{6}\) There are different reviews on record focusing on HIF-1\(\alpha\) targeted therapeutic anticancer strategies.\(^{6,9}\) These articles describe different methods of silencing the HIF-1\(\alpha\) expression.\(^{6,9}\) Small molecule inhibitors such as the mTOR inhibitor rapamycin seem to be the most effective strategy to block HIF-1\(\alpha\) activity.\(^{6}\) A number of other substances including the topoisomerase I inhibitor topotecan, inhibitors of farnesyl transferase, VEGFR and Raf inhibitors or vincristine and 2-methoxyestradiol seem to block the activity of HIF-1\(\alpha\).\(^{6,9}\) Nevertheless, further studies focusing on reagents that are capable of reducing HIF-1\(\alpha\) levels in MCC are needed.

Glut-1 is overexpressed in a variety of cancer types and this overexpression is assumed to provide an increased amount of

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**Table 3** (a) and (b) Correlation between the different protein expression scores as indicated by Spearman’s rank correlation coefficients

|     | HIF-1\(\alpha\) | VEGFR-3 | Glut-1 | MCT4 | CAIX |
|-----|---------------|---------|--------|------|------|
| (a) | McPyV\(^{+}\) tumours |
| HIF-1\(\alpha\) | 1 | 0.160 | 0.180 | 0.466 | 0.005 |
| VEGFR-3 | 0.160 | 1 | 0.203 | 0.336 | 0.356 |
| Glut-1 | 0.180 | 0.203 | 1 | 0.307 | 0.044 |
| MCT4 | 0.466 | 0.356 | 0.307 | 1 | 0.313 |
| CAIX | 0.005 | 0.356 | -0.313 | -0.044 | 1 |
| (b) | McPyV\(^{-}\) tumours |
| HIF-1\(\alpha\) | 1 | 0.703 | 0.913* | 0.211 | -0.054 |
| VEGFR-3 | 0.703 | 1 | 0.740 | 0.051 | 0.158 |
| Glut-1 | 0.913* | 0.740 | 1 | 0.289 | 0.148 |
| MCT4 | 0.211 | 0.051 | 0.289 | 1 | 0.872 |
| CAIX | -0.054 | 0.158 | 0.148 | 0.872 | 1 |

McPyV, Merkel cell polyomavirus.

*\( P < 0.05 \).
glucose that is needed in proliferating tumour cells. Nevertheless, studies analysing the Glut-1 expression in skin cancer are rare and reveal conflicting results regarding the diagnostic and prognostic impact of Glut-1 expression. Glut-1 was expressed in 91% of the McPyV+ samples and in 60% of the McPyV- tumours. Glut-1 expression in both groups was predominantly found in the peripheral parts of the tumours. Besides our study, there is only one study on Glut-1 expression in MCC showing that Glut-1 expression (ranging from 10% to 86.7% of tumour cells) was positively correlated with fluorodeoxyglucose uptake in positron emission tomography. Our data provide evidence that HIF-1α-induced upregulation of Glut-1 contributes to metabolic reprogramming in MCC; further studies focusing on Glut-1 expression in MCC and its prognostic impact are thus warranted.

In the present study, we found MCT4 to be expressed in both McPyV+ MCC (91%) and McPyV- MCC (80%). An important function of monocarboxylate transporters including MCT4 is to prevent the toxic build-up of intracellular lactate by mediating the efflux of lactate together with protons. MCT4 expression in melanoma is reported to be higher than that in nevi and is associated with tumour thickness, mitotic index, higher pT and pN, as well as with nodular histotype, locoregional recurrence and shorter overall survival. Berrios et al. showed that MCT1 upregulation is driven by the McPyV small T antigen and that inhibition of MCT1 activity can suppress MCC growth. MCT4 positive cells in our samples were predominantly present in tumour areas with Glut-1 overexpression. These findings seem plausible as overexpression of Glut-1 enables tumour cells to uptake increased amounts of glucose. Consecutively, increased glycolysis leads to higher intracellular lactate levels that can be equalized by the increased activity and expression of MCT4. The expression of MCT4 and Glut-1 was positively correlated in McPyV+ and McPyV- although their correlation was not statistically significant. Nevertheless, our study is the first to analyse MCT4 expression in MCC; further studies are thus necessary to gain more insight into the pathogenic role and potential therapeutic implications of MCT4 in MCC.

CAIX, a HIF-1α inducible pH regulator, was expressed in all of the investigated samples irrespective of the McPyV status indicating that CAIX is upregulated in MCC, independently of the McPyV status.

Enhanced glucose uptake and its metabolism to lactic acid by cancer cells results in acidosis (low pH) which disrupts proliferation, migration, invasion and metastasis. CAIX contributes to pH homeostasis under hypoxic conditions, and its membrane-bound overexpression has been described in various human malignancies including breast carcinoma, renal cell carcinoma, adenocarcinoma of the cervix and colon, lung carcinoma, and malignant melanoma, and has often been associated with poor outcome. Analogous to the distribution of HIF-1α, Glut-1 and MCT4, CAIX tends to be more strongly expressed in the invading edges of tumour margins. Further studies on the metabolic signature of MCC should also focus on CAIX and its prognostic impact.

VEGF, an important downstream target of HIF-1α, plays a central role in physiologic and pathologic (lymph-) angiogenesis and may additionally act as a potential therapeutic target. VEGF exerts paracrine effects on endothelial cells but may also influence skin carcinogenesis by altering the survival, proliferation or stemness of tumour cells via an autocrine loop. Only few studies have analysed the expression of VEGF and its receptors in MCC. Interestingly, we found no tumoural VEGF expression in the investigated MCC samples irrespective of the McPyV status, indicating that VEGF expression by tumour cells does not play a crucial role in MCC pathogenesis. These findings are in accordance with an earlier work by our group demonstrating that the main source of VEGF production in MCC seems to be tumour-associated macrophages.

Kase et al. described a series of MCC samples of the eyelid that were also negative for VEGF. Nevertheless, epidermal VEGF expression was present in all of our MCC samples. Interestingly, in most cases the highest epidermal VEGF levels were present in the epithelium directly above the MCC. This strong epidermal VEGF expression suggests an interaction between MCC and the overlying epidermis. Cytokines such as transforming growth factor alpha released by tumour cells may stimulate VEGF production by epidermal keratinocytes. Epidermal VEGF production may consecutively lead to angiogenesis via paracrine effects on endothelial cells and may additionally stimulate proliferation, maintain stemness and promote the survival of tumours through direct effects on tumour cells.

Only few studies have analysed VEGFR-2 or VEGFR-3 expression in MCC. We found no VEGFR-2 expression in tumour cells, whereas VEGFR-3 was expressed in all McPyV+ and McPyV- MCC samples. These findings indicate a minor role of VEGFR-2 compared to that of VEGFR-3 in MCC pathogenesis. Our findings on VEGFR-2 expression are in contrast with the results of Kukko et al. reporting VEGFR-2 expression in 70–91% of investigated MCC samples according to the tumour size and of Brunner et al. describing VEGFR-2 positivity in 88% of MCC samples. These discrepancies might be explained by differences in the investigated study populations, variations in antibody specificity and by the definition of VEGFR-2 positivity. Remarkably, Kukko et al. considered the tumour positive for VEGFR-2 irrespective of the source of VEGFR-2 (tumour cells, stroma or vasculature), whereas we only rated a sample as positive when VEGFR-2 was expressed by tumour cells.

Taken together, HIF-1α and its metabolic downstream targets Glut-1, MCT4 and CAIX were predominantly upregulated in peripheral areas of the analysed MCC samples. This expression profile may reflect the hypoxic conditions in the rapidly proliferating cells at the tumour periphery that are not (yet) supported...
by a well-established tumour vasculature. Furthermore, although tumour VEGF production was not present in the investigated MCC samples, we found a strong expression of VEGFR-3 by tumour cells in the majority of the analysed MCC samples.

Despite some limitations (i.e. retrospective analysis, relatively small study population, no clinical follow-up data), our study provides for the first time, strong evidence that the expression of HIF-1α, Glut-1, MCT4, CAIX and VEGFR-3 may play a critical role in the pathogenesis of MCC and that tumours with positive McPyV status and negative McPyV status show differences in their metabolic signature.

Thus, our observations improve the knowledge on the metabolic signature of MCC. This might have implications for new treatment modalities targeting HIF-1α and/or its downstream factors. Nevertheless, prospective multicentre studies, investigating a higher number of tumours and including follow-up data, are desirable, to confirm the current findings.

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