We now can return to the theme of bioengineering stem cells to form hair follicles. With the advent of stem cell biology, we anticipate that many attempts to engineer hair follicles will be made in the near future. The epidermal stem cells or progenitor cells may derive from many sources: embryonic stem cells, engineered embryonic stem cells or cell lines, interfollicular epidermal stem cells, bulge stem cells, or even bone marrow stem cells. Because the cells may have different competences, the "hair follicles" built with them may be different. In the future, we must ask whether they fulfill all the criteria of a bona fide hair follicle. Work that has had partial success is nonetheless valuable because it helps in the analysis of distinct events during hair-follicle morphogenesis. The results here highlight the challenge faced by all who aspire to engineer human organs, including hair follicles.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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See related article on pg 2116

Merkel Cell Carcinoma: More Deaths but Still No Pathway to Blame

Bianca Lemos[1] and Paul Nghiem[1]

Merkel cell carcinoma (MCC) is a neuroendocrine skin cancer with a rising incidence (1500 U.S. cases per year) that now exceeds that of cutaneous T-cell lymphoma and a mortality (33%) exceeding that of melanoma. Despite this impact, little is known about its biology. Recent studies have shown that Ras/MAP kinase activity is absent and possibly detrimental to this cancer. This makes MCC distinct from other UV-induced skin cancers and highlights the question of what drives this malignancy.

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Merkel cell carcinoma (MCC) is an aggressive neuroendocrine carcinoma of the skin associated with ultraviolet exposure. Although uncommon, its incidence is increasing and has in fact tripled in the past 15 years (Hodgson, 2005). The rise in incidence is due in part to improved diagnosis through the

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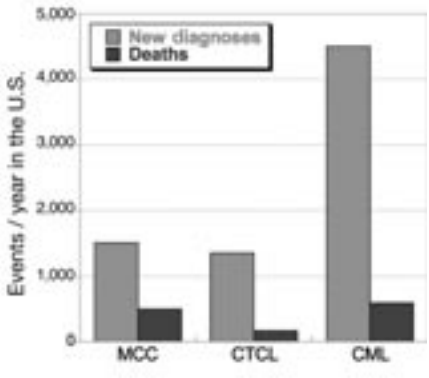

Figure 1. Annual incidence and mortality for three cancers in the United States. MCC incidence is similar to that of CTCL, and MCC deaths are similar to those of CML. Abbreviations: MCC, Merkel cell carcinoma; CTCL, cutaneous T cell lymphoma; CML, chronic myelogenous leukemia. (Data from American Cancer Society, 2006; Weinstock and Gardstein, 1999.)

routine use of cytokeratin-20 staining but is also due to an aging population with extensive sun exposure. The National Cancer Data Base, a registry that captures approximately 75% of all cancer cases in the United States, recorded 986 cases of MCC in 2004 (most recent data available). According to multiple national databases, there has been a 5–10% annual increase in MCC diagnoses beginning in the early 1990s (Agelli and Clegg, 2003; National Cancer Data Base data provided by Jerri Linn Phillips). Taking these factors into account, there are now approximately 1,500 cases of MCC per year in the United States. This exceeds the incidence of cutaneous T-cell lymphoma, a well-known and frequently studied cancer, whose incidence has been stable since the early 1980s (Weinstock and Gardstein, 1999). MCC is also one of the most lethal skin cancers, with a 33% mortality (Hodgson, 2005)—it now kills more patients than cutaneous T-cell lymphoma and a number similar to that for chronic myelogenous leukemia (Figure 1) (American Cancer Society, 2006; Weinstock and Gardstein, 1999).

Despite the mortality and increasing incidence, virtually nothing is known about the molecular basis of MCC. Several studies of oncogenic pathways have been carried out in MCC but have not elucidated a significant role for these pathways in this cancer (Table 1). Van Gele *et al.* (2000) revealed a lack of p53 mutations in 12 of 15 cases of MCC. Three of the five p53 mutations found in three tumors were of the signature UV type, consistent with MCC's known association with UV exposure. The lack of p53 mutations in 80% of MCCs, however, suggests that p53 is not typically involved in this cancer. The same group (Van Gele *et al.*, 2001) also looked at the role of the tumor suppressor *PTEN*. Although loss of heterozygosity (loss of one allele) was seen in 9 of 21 cases (mostly involving loss of the entire arm of chromosome 10), only a single mutation was seen in the remaining allele of one MCC case. Thus, inactivation of *PTEN* (by loss of one allele and disruption of the second) does not play a frequent role in MCC oncogenesis. It is possible, however, that loss of a second *PTEN* allele would be via an epigenetic silencing mechanism such as methylation, which has not been examined.

The *Wnt* pathway and its role in MCC have also been evaluated by looking for nuclear accumulation of β-catenin and for mutations in β-catenin and three other related genes. In a series of 12 MCC cases, Liu *et al.* (2007) found elevated β-catenin accumulation in only one tumor and no mutations in any tumors and concluded that the *Wnt* pathway is not implicated in MCC. Several studies have reported on c-Kit expression in MCC tumor samples. Most recently, Swick *et al.* (2007) determined that although eight of nine MCC tumors were positive for c-Kit by immunohistochemical staining, no activating mutations were present in the four exons commonly found to have mutations in this gene.

> The MAP kinase pathway is silent in 42 of 44 MCC tumors.

MAP kinase signaling, a common feature of many epithelial cancers, is the most studied oncogenic pathway in MCC (Figure 2). Popp *et al.* (2002) looked for activating mutations in exons 1 and 2 of the *H-*, *K-*, and *N-ras* genes in six MCC cell lines but found none.

Table 1. Cancer-associated pathways and genes studied in MCC oncogenesis

Cancer-associated pathway/gene	Likely relevant	Summary of findings	Reference
p53	–	No mutations found in 12 of 15 samples	Van Gele *et al.*, 2000
Ras	–	No activating mutations in *H-ras*, *K-ras*, or *N-ras* found in six MCC cell lines	Popp *et al.*, 2002
B-Raf^{V600E}	–	No mutations in 46 MCCs	Houben *et al.*, 2006
MAP kinase activity	–	MAP kinase silenced in 42/44 MCCs	Houben *et al.*, 2006
Wnt	–	No mutations in β-catenin, APC, AXIN1, or AXIN2 in 12 MCC tumors	Liu *et al.*, 2007
c-Kit	–	No activating mutations in nine MCC tumors	Swick *et al.*, 2007
PTEN	?	No mutations in 20 of 21 samples but loss of heterozygosity for region in 43%	Van Gele *et al.*, 2001
bcl-2	+	High expression in 15 of 20 MCC tumors; bcl-2 antisense decreases tumor size in xenograft model	Kennedy *et al.*, 1996; Plettenberg *et al.*, 1996; Schlagbauer-Wadl *et al.*, 2000

Recently, Houben *et al.* (2006) evaluated 46 MCC tumors for the characteristic activating B-Raf mutation (V600E) found in 43% of melanomas, and all tumors were negative. Further, the authors showed that the MAP kinase pathway was silent (no phospho-ERK staining) in 42 of 44 cases, and *Raf* kinase inhibitor protein was increased in all 20 tumors evaluated. Taken together, the studies strongly suggest no role for MAP kinase pathway activation in MCC, a pathway heavily implicated in the etiology of melanoma, squamous cell carcinoma, and many other cancers (Schubbert *et al.*, 2007).

The sole potentially positive finding in MCC is that of bcl-2. Its expression was seen in 15 of 20 MCC tumors in two studies (Kennedy *et al.*, 1996; Plettenberg *et al.*, 1996). A separate study determined that decreasing bcl-2 expression *in vivo* by systemic antisense oligonucleotide (oblimersen/Genasense) administration in a SCID mouse/human tumor xenograft model resulted in tumor shrinkage (Schlagbauer-Wadl *et al.*, 2000). The expression of this anti-apoptosis protein is a common finding in many cancers and suggests one of its mechanisms to avoid cell death; however, it does not illuminate the promitotic pathways that drive MCC.

In this issue of the *Journal of Investigative Dermatology*, Houben *et al.* (2007) expand on their findings noted above, that the MAP kinase pathway is silent in 42 of 44 MCC tumors. Using an MCC cell line (UISO) that, like primary tumors, shows no evidence of MAP kinase activity, they tested whether activating this pathway could result in MCC cell death. Indeed, inducible expression of c-Raf-1 kinase in the UISO MCC cell line resulted in morphologic changes and apoptosis. Rounding up and detachment of cells, development of long cytoplasmic extensions, and loss of actin stress fibers were seen beginning at 10 hours after activation of the pathway. Further, they demonstrated that active Raf kinase induced apoptosis in UISO cells in a time- and dose-dependent manner. This appears to be via a classic apoptotic pathway because it was blocked by a caspase inhibitor (Z-VAD-FMK). The fact that a MEK kinase inbibitor (U0126) also blocked Raf's ability to induce apoptosis suggests that the Raf effect is indeed mediated via the predicted MAP kinase pathway (Houben *et al.*, 2007).

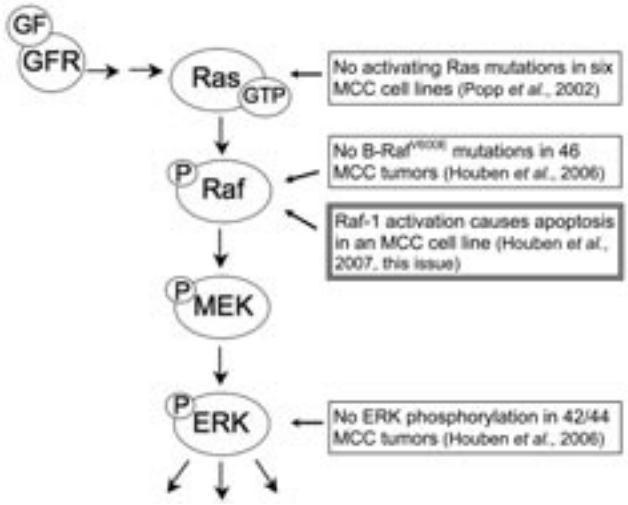

Figure 2. The MAP kinase pathway. Text boxes annotate study findings of relevance in MCC. Double box indicates findings from study in this issue. (Adapted from Houben *et al.*, 2006.)

A limitation of the study is that it was performed in a single MCC cell line. Few reagents for MCC are available to study, and of the available cell lines, only one (UISO) had preservation of the MCC *in vivo* characteristic of silencing of the MAP kinase pathway. The lack of cell death observed in the study from Raf overexpression in other cell lines positive for MAP kinase activity (three melanoma lines and two other MCC lines) argues for some level of specificity for the cell-death effect to cells that are silent for MAP kinase activity.

The striking lack of knowledge of what drives MCC oncogenesis is an important impediment because no effective targeted therapies exist. Molecular therapies used in other malignancies are now being extended to clinical trials in MCC. Phase II trials of imatinib (Gleevec) and oblimersen (Genasense) are currently under way in MCC. The tyrosine kinase inhibitor imatinib, initially designed to target the Bcr–Abl translocation in chronic myelogenous leukemia, is being explored because of the c-Kit positivity of MCC. Recent data from Swick *et al.* (2007) suggest that its effectiveness may be limited, however, given that no c-Kit-activating mutations were seen in their series. The bcl-2 inhibitor oblimersen is also in a phase II trial for MCC, but no results of its efficacy in humans with MCC have been published.

The present study by Houben *et al.* (2007) provides data for the possible use of MAP kinase pathway–activating agents in MCC. Use of the Raf-1 activator ZM336372 did indeed induce ERK phosphylation in UISO MCC cells that are normally silent for MAP kinase signaling. A separate study using carcinoid tumor cells (another neuroendocrine carcinoma) demonstrated that *in vitro* treatment with ZM336372 inhibited cellular proliferation (Van Gompel *et al.*, 2005). The data suggest that pharmacologic activation of this normally silent pathway is a potential target for treatment of certain neuroendocrine tumors.

Even though we know essentially nothing about what promitotic signaling mechanisms drive MCC, there is a glimmer of hope that recent knowledge about what does *not* drive it may be brought to bear in the fight against MCC and, potentially, other neuroendocrine cancers.

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CONFLICT OF INTEREST

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How Different Wavelengths of the Ultraviolet Spectrum Contribute to Skin Carcinogenesis: The Role of Cellular Damage Responses

Thomas M. Rünger[1]

The carcinogenic properties of ultraviolet (UV) light are mediated by its ability to generate DNA damage. Cellular responses to UV-induced DNA damage profoundly modulate the carcinogenic effects of UV exposures, and these responses are wavelength dependent. However, the exact contributions of different wavelengths of UV light to DNA damage, cellular damage responses, mutation, and skin carcinogenesis are incompletely understood. Given that UV-induced apoptosis is a protective cellular response to UV that prevents survival of damaged cells, inhibition of UVB-induced apoptosis by adding UVA, as reported by Ibuki *et al*. in this issue, may be a mechanism by which UVA augments UVB-mediated mutation and skin cancer formation.

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Photocarcinogenesis is most commonly thought to be the result of a chain of events that involve formation of DNA damage and subsequent mutation formation following exposure to ultraviolet light (UV) (Figure 1) (Rünger, 2003). Several cellular defense mechanisms reduce the likelihood that one occurrence in this cascade leads to the next (Figure 1). Many of these protective responses can be induced by UV, thereby providing additional protection against subsequent exposures. All of these responses are mediated by a tightly controlled network of signaling pathways, many of which involve the tumor suppressor p53. UV-induced mutations of p53 with subsequent disruption of cellular damage responses are a hallmark of most sun-induced cutaneous squamous-cell carcinomas (Brash *et al*., 1996). This further underlines the importance of these damage responses for the prevention of photocarcinogenesis.

The melanin microparasol that covers the nucleus of basal keratinocytes reduces formation of DNA damage with exposure of the skin to UV. Once DNA damage is formed, most of it is repaired and does not give rise to mutations. This is exemplified by the genodermatosis xeroderma pigmentosum, in which the relative inability to repair UV-induced DNA damage results in UV hypermutability in cells and an increased frequency of skin cancers in UV-exposed areas of affected patients. Another mechanism that

> UVA radiation inhibits UVB-induced apoptosis in mouse epidermal cells.

inhibits mutation formation at sites of DNA damage is a G1/S cell–cycle arrest that prevents cells from replicating damaged DNA, a situation particularly prone to the introduction of mutations (Decraene *et al*., 2001). DNA polymerase η, which is mutated in xeroderma pigmentosum variant, is a translesion DNA polymerase spe-

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