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Akt mediates an angiogenic switch in transformed keratinocytes

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Akt signaling is involved in tumorigenesis via a number of different mechanisms that result in increased proliferation and decreased apoptosis. Previous data have demonstrated that Akt-mediated signaling is functionally involved in keratinocyte transformation. This work investigates the involvement of angiogenesis as a mediator of tumorigenesis in Akt-transformed keratinocytes. Tumors produced by subcutaneous injection of the latter showed increased angiogenic profiles associated with increased vascular endothelial growth factor (VEGF) protein levels. However, in contrast to v-ras^{Ha}-transformed keratinocytes, VEGF mRNA levels were not increased. The induction of VEGF protein by Akt is associated with increased phosphorylation and thus activation of p70S6K and eIF4E-binding protein 1, leading to increased VEGF translation. In addition, we observed increased metaloproteinases 2 and 9 expression, but not thrombospondin 1, in tumors derived from Akt-transformed keratinocytes. Collectively, these results demonstrate that Akt is an important mediator of angiogenesis in malignant keratinocytes through a post-transcriptional mechanism.

Introduction

The mouse skin carcinogenesis model has provided an important instrumental framework for understanding many of the concepts currently applied to human neoplasia (reviewed in refs 1,2). The importance of Akt-dependent signaling in mouse skin tumorigenesis has recently been demonstrated as Akt activity increases in parallel with the process of tumor progression and precedes that of MAPK/ERK (3). In addition, over-expression of Akt, which leads to increased Akt activity, exacerbates the tumorigenic behavior of murine keratinocytes (increased proliferation, decreased apoptosis and impaired differentiation) (3). In agreement, specific ablation of PTEN tumor suppressor gene in the epidermis leads to the formation of spontaneous epidermal tumors and increased sensitivity to chemical mouse skin carcinogenesis (4). Further, in experiments with transgenic mice, the ectopic expression of keratin K10—which inhibits Akt activation (5,6)—also results in dramatic inhibition of tumor development (5,7). These observations are in agreement with the current view that Akt functions in tumorigenesis in association with increased proliferation and survival of the transformed cells (8,9).

Besides alterations in proliferation and apoptosis, the induction of angiogenesis is essential in tumor growth since the generation of new vessels allows rapid tumor expansion and increases the likelihood of metastatic events. The acquisition of the angiogenic phenotype during tumorigenesis, the so-called angiogenic switch (10), is thought to be induced by a change in the balance of positive and negative regulators of endothelial cell growth (10). Among these, vascular endothelial growth factor (VEGF) is thought to be one of the major angiogenesis factors in malignant tumor growth (11–13).

In the mouse skin carcinogenesis model, angiogenesis is an early event. The development of papillomas is preceded by a burst of angiogenesis (14). In addition, the activation of Ha-ras, the major critical event in tumor initiation in this system, plays a major role in the tumor angiogenic response inducing VEGF expression (15,16). The importance of VEGF in mouse skin carcinogenesis is demonstrated by accelerated tumor development in transgenic mice expressing VEGF (15), and by the rescue of tumor growth inhibition caused by functional EGFR abrogation promoted by VEGF expression (17). In addition to VEGF, other important factors such matrix metaloproteinases 2 (MMP2) and 9 (MMP9), thrombospondins 1 and 2 (TSP1, TSP2) have also recently emerged as important regulators of tumoral angiogenesis in mouse skin carcinogenesis (18–24).

There is evidence that suggests the PI3K/PTEN/Akt pathway may be involved in tumor angiogenesis (reviewed in ref. 25). This appears to proceed mainly through the regulation of VEGF expression (26–28) and TSP1 (29). However, the precise involvement of the different elements of the pathway and the molecular mechanisms resulting in the angiogenic response are not well understood. Given the reported essential role of Akt in mouse keratinocyte transformation (3), the aim of the present study was to investigate whether Akt signaling also regulated tumor angiogenesis in this system.

The results show that Akt modulates the angiogenic profile and VEGF up-regulation by a post-transcriptional mechanism associated with increased p70S6K and eIF4E-binding protein 1 (4E-BP1) phosphorylation. Further, Akt-induced tumors also show increased MMP2 and MMP9 expression, but no alteration in TSP1. This provides evidence that Akt plays a central role in the establishment of stromal changes leading to skin tumoral growth.

Materials and methods

Cell culture, transfection and in vivo tumorigenic assays

Mouse PB keratinocytes (30) and Akt-transfected derivatives (as 40–80 pooled clones) were grown and subcutaneously injected into *nu*/*nu* mice as reported

Abbreviations: HIF1α, hypoxia-inducible factor 1; MMP2, metaloproteinase 2; MMP9, metaloproteinase 9; TSP1, thrombospondin 1; TSP2 thrombospondin 2; VEGF, vascular endothelial growth factor.