SWI/SNF complex activates the expression of survival genes in melanoma

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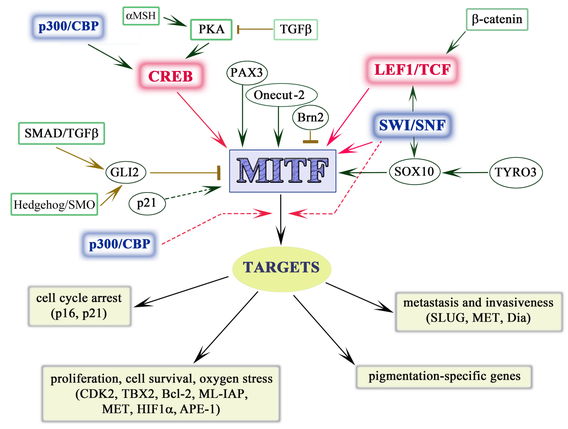
INTRODUCTION

SWI/SNF chromatin remodeling complex is multisubunit protein machine which is capable of changing the local structure of chromatin and is present in cells with only subtle differences in subunit composition. The main two types of SWI/SNF complexes are characterized by the presence of an ATPase subunit, which is either Brm (brahma) or Brg1 (brahma related gene 1).

It is known that loss of Brm or Brg1 is implicated in cancer progression. However, SWI/SNF may behave also as a tumor promoter, depending on the cancer tissue context.

Malignant melanoma is highly invasive and early metastasizing tumor and is known for its resistance to convential anticancer therapies. Microphthalmia-associated transcription factor (MITF), pivotal transcriptional regulator of normal and malignat melanocytes, requires SWI/SNF complex for the expression of its downstream genes as well as for its own expression. These findings placed SWI/SNF together with MITF on the central crossroad in the melanoma transcriptional network, influencing the basic processes of melanoma biology.

Components of SWI/SNF are generally well expressed in malignant melanocytes and at least one ATPase is always present in melanoma cell lines. Here we demonstrate that Brg1 and the SWI/SNF complex are involved in melanoma progression and possibly metastasis through the MITF-dependent and MITF-independent mechanisms and suggest the disparity of the SWI/SNF function in different types of cancer cells.



RESULTS

Both ATPases are expressed in melanomas.

Whereas Brg1 expression might be attenuated or rarely entirely lost in some tumor cells, Brm positivity is maintained in nevi and primary melanomas. MITF-M immunostaining showed heterogenity of expression in nevi and melanomas with some cells being devoid of MITF-M expression (Figure 1).

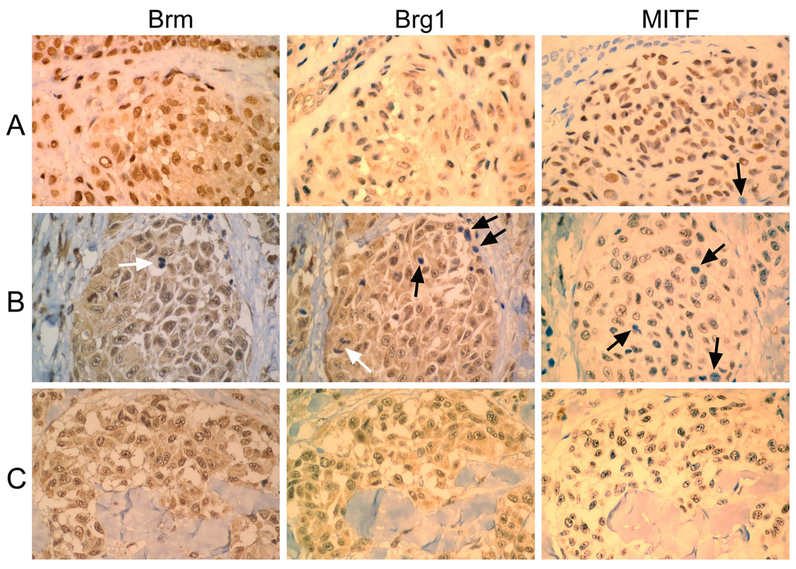


Fig.1 Immunohistochemical analysis
Parallel sections (5 mm) were stained with antibodies against Brm (left), Brg1 (middle), and MITF (right). A, intradermal nevus; B,C, primary melanomas. Protein expression was analyzed in intradermal or compound nevi (5 sections) and primary melanomas >1 mm in thickness (9 sections) and representative images were shown. Black arrows indicate negative interphase nuclei in Brg1 and MITF staining, open arrows mark Brm- or Brg1-negative mitotic nuclei. Magnification, x400.

Both Brg1 or MITF depletion severely reduced proliferation of 501mel cells and depletion of Brm in Brg1-negative SK-MEL-5 cells led to the block of proliferation (Figure 2).

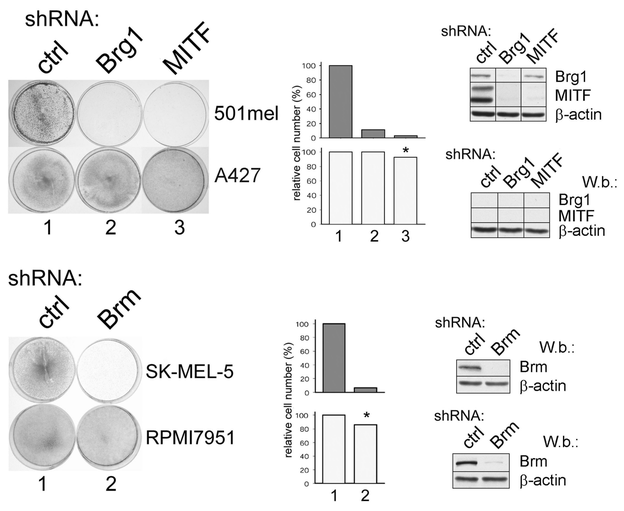


Fig.2 Colony formation
Top, Brg1 or MITF-M depletion inhibits colony formation in 501mel cells. A427 and RPMI7951 cells were used as controls; bottom, Brm depletion inhibits cell growth in Brg1 negative SK-MEL-5 cells. Western blot confirms the efficiency of Brg1, Brm and MITF knockdown in the cells. *, not statistically significant (P<0.01).

Brg1 or Brm may function as cofactors for transcription of MITF in melanoma cells.

Brm bound to the proximal region of the MITF promoter in Brg1-negative SK-MEL-5 cells. By contrast, Brm/Brg1-positive melanoma cells, 501mel and Hbl, displayed only Brg1 occupancy. Thus, the CHIP results suggest that Brg1 is the primary ATPase recruited to MITF promoter in Brm/Brg1-positive melanoma cells while Brm occupies the promoter if Brg1 is lost.

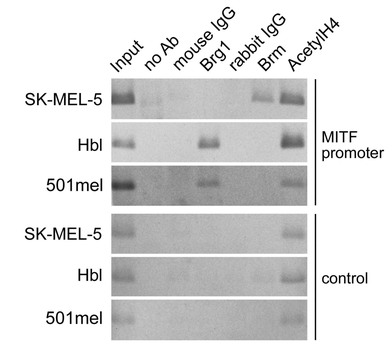


Fig. 3 Chromatinimmunoprecipitation
Chromatin immunoprecipitations from SK-MEL-5, 501mel and Hbl cells are shown. Brg1 or Brm binds to the endogenous MITF promoter in melanoma cells.

Brg1 is required for the maintainance of pigment cell-specific transcription regulated by MITF-M.

Expression of 381 genes (represented by 485 probes) had been downregulated >2-fold (P<0.005) when Brg1 was depleted and these include many MITF targets. Other MITF targets were decreased to a lesser extent. By contrast, a smaller number of genes (210, represented by 273 probes) was upregulated >2-fold (P<0.005).

Prosurvival molecules are decreased in Brg1-depleted 501mel cells

Further, expression of several anti-apoptotic, proliferative and pro-invasive factors in cancer, for example insulin growth factor 1 (IGF1), osteopontin (OPN), TGFβ2, were also strongly downregulated. To validate the microarray results, the protein and mRNA expression was estimated and was found that protein levels of OPN, survivin and also livin, a MITF-M transcriptional target, were substantially decreased in Brg1-depleted cells (Figure 5a, 5c). The mRNA levels of OPN, survivin, and IGF1 were also significantly reduced, by RT-PCR (Figure 5b). This effect is through the MITF-independent mechanism as shows figure 5d. Protein levels of these proteins remained unchanged in MITF-depleted cells.

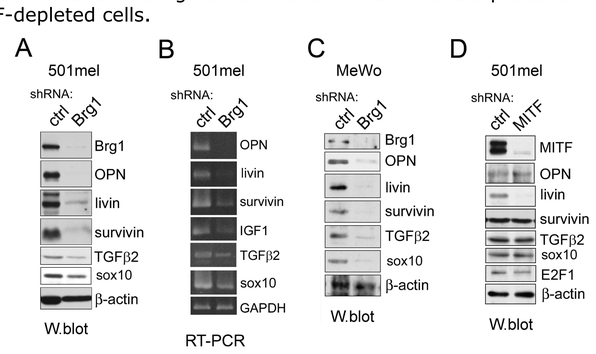


Fig.5 Western blot and RT-PCR
a) Protein expression of OPN, livin, survivin, TGFβ2 and Sox10 after Brg1 silencing in 501mel cells
b) Decreased mRNA levels for OPN, livin, survivin, TGFβ2 and Sox10 after Brg1 silencing in 501mel cells
c) Protein expression of OPN, livin, survivin, TGFβ2 and Sox10 after Brg1 silencing in MeWo cells
d) Protein expression of OPN, livin, survivin, TGFβ2 and Sox10 after MITF silencing in 501mel cells

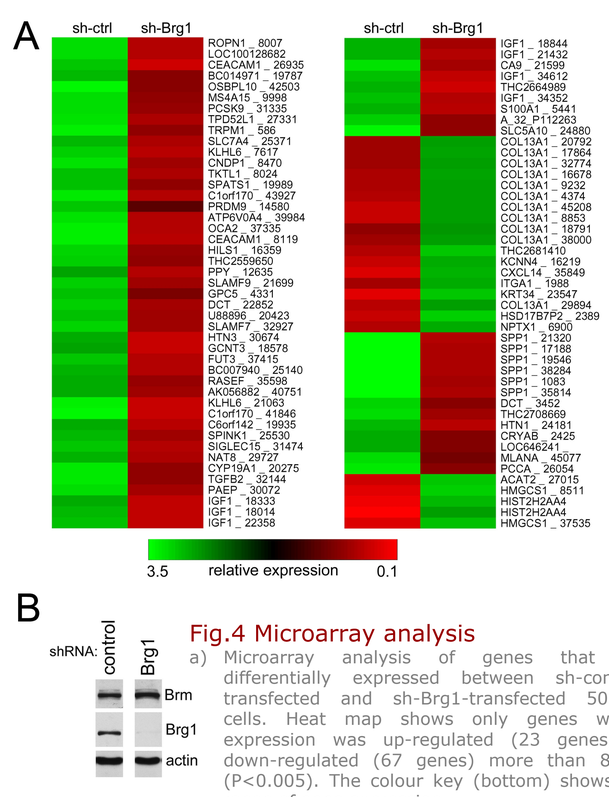


Fig.4 Microarray analysis
a) Microarray analysis of genes that are differentially expressed between sh-control-transfected and sh-Brg1-transfected 501mel cells. Heat map shows only genes whose expression was up-regulated (23 genes) or down-regulated (67 genes) more than 8-fold (P<0.005). The colour key (bottom) shows the range of gene expression.
b) Western blot confirming the efficiency of Brg1 knockdown in the cells which were used for microarray analysis. Brm was not appreciably affected.

Cell proliferation was partly rescued by CDK2, osteopontin, and IGF1 in Brg1-depleted melanoma cells.

Next, we explored the consequences of overexpressing MITF-M and several prosurvival proteins on proliferation of Brg1-depleted cells.

- Addition of recombinant OPN protein alone rescued the colony formation by about ~2-fold (Figure 6a), whereas individually overexpressed livin, survivin, or Bcl-2 failed to show any effect (data not shown).
- Overexpression of the MITF-Vp16 chimera, a transcriptionally more active MITF-M derivative, only insignificantly increased the number of colonies, whereas the MITF-M target CDK2 was more effective (Figure 6b).
- Furthermore, the presence of IGF1 protein in the culture medium increased proliferation of Brg1-blocked cells in a dose-dependent manner up to about 3~fold when compared with nontreated cells (Figure 6c).

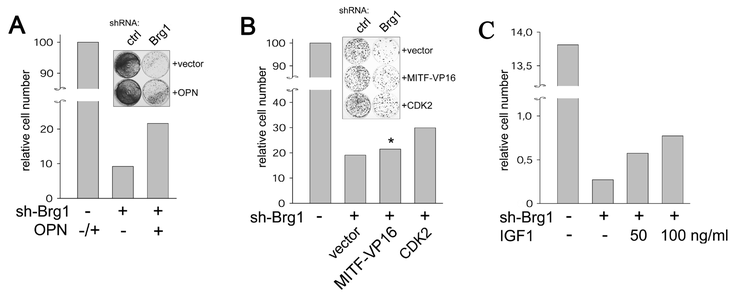


Fig.6 Colony formation
a) Overexpression of recombinant OPN (50 mg/ml) can partly rescue colony formation of Brg1-silenced 501mel cells.
b) Overexpression of CDK2, but not MITF-VP16 chimera, partly rescues colony formation of Brg1-silenced cells. *, not statistically significant (P<0.05), compared to control.
c) Exogenously added IGF1 plasmid mildly restores colony formation of Brg1-depleted 501mel cells.

CONCLUSION

- **SWI/SNF complex is not only a coactivator of expression of many MITF target genes, but it is also an essential cofactor for transcription of MITF itself in melanoma cells.**
- **At least one ATPase expression is necessary for MITF expression in melanoma cells.**
- **The prosurvival role of Brg1 can be provided, besides activating the MITF axis, also through MITF-independent mechanims in melanoma.**
- **A tissue-aimed inactivation of the SWI/SNF complex might become an effective approach in the therapy of melanoma.**

Grant support: NT/11229-3, IGA MH, Czech Republic.