phorylation of the carboxy-terminal tail by the atypical PKC (aPKC) prevents 20S proteasomal degradation of SRC-3, negating the effects of p38 MAP kinase and GSK-3 activity. The net result of aPKC phosphorylation is the cellular accumulation of the coactivator and enhanced ER-dependent gene transcription. Interestingly, other NRs such as the progesterone receptor do not exhibit a similar ability to trigger phosphorylation of SRC-3 by aPKC, highlighting the relevance of this mechanism to estrogen-dependent breast cancer cell growth. The authors show that the normal degradation of SRC-3 involves an initial interaction with the C8 alpha component of the 20S proteasome. However, when hyperphosphorylated by aPKC, SRC-3 undergoes a conformational change, exposing a negatively charged sequence that inhibits the interaction with C8 via electrostatic repulsion, effectively shielding it from the proteasome and resulting in a net increase in the cellular pool of this cofactor.

The relevance of this finding to breast cancer pathology is strengthened by the observation that SRC-3 phosphorylation by aPKC is promoted by hormone-activated ER. This leads to a proposal that estradiol binding to ER allows the receptor to associate and stabilize the SRC-3-aPKC dimer (Figure 1). The presumptive trimeric complex facilitates SRC-3 phosphorylation, which in turn increases transcription of ER of target genes. Interestingly, overexpression of aPKC has been observed in a number of cancers (Regala et al., 2005), which may provide an independent means to enhance SRC-3 pool size and ER transcription. Beyond NRs, SRC-3 has been shown to be a coactivator of other transcriptional networks, including activator protein-1 (AP-1), nuclear factor-κB (NFκB), signal transducer and activator of transcription (STAT), and E2F1, all of which have been associated with cell growth and cancer (Liao et al., 2002). Thus, the work presented by Yi and colleagues provides us with a new model of how posttranslation modifications of a NR coactivator may promote cancer while offering a new therapeutic target to exploit in its treatment.

REFERENCES

American Cancer Society. (2008). Cancer Facts and Figures (http://www.cancer.org/downloads/STT/2008CAFFfinalsecured.pdf).

Anzick, S.L., Kononen, J., Walker, R.L., Azorsa, D.O., Tanner, M.M., Guan, X.Y., Sauter, G., Kallioniemi, O.P., Trent, J.M., and Meltzer, P.S. (1997). Science *277*, 965–968.

Chen, H., Lin, R.J., Schiltz, R.L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M.L., Nakatani, Y., and Evans, R.M. (1997). Cell *90*, 569–580.

Chen, H., Lin, R.J., Xie, W., Wilpitz, D., and Evans, R.M. (1999). Cell *98*, 675–686.

Liao, L., Kuang, S.Q., Yuan, Y., Gonzalez, S.M., O'Malley, B.W., and Xu, J. (2002). J. Steroid Biochem. Mol. Biol. *83*, 3–14.

Puigserver, P., and Spiegelman, B.M. (2003). Endocr. Rev. *24*, 78–90.

Regala, R.P., Weems, C., Jamieson, L., Khoor, A., Edell, E.S., Lohse, C.M., and Fields, A.P. (2005). Cancer Res. *65*, 8905–8911.

Torchia, J., Rose, D.W., Inostroza, J., Kamei, Y., Westin, S., Glass, C.K., and Rosenfeld, M.G. (1997). Nature *387*, 677–684.

Wu, R.C., Feng, Q., Lonard, D.M., and O'Malley, B.W. (2007). Cell *129*, 1125–1140.

Yi, P., Feng, Q., Amazit, L., Lonard, D.M., Tsai, S.Y., Tsai, M.-J., and O'Malley, B.W. (2008). Mol. Cell. *29*, 465–476.

Learning the ABCs of Melanoma-Initiating Cells

Susan E. Zabierowski[1] and Meenhard Herlyn[1,*]
[1]The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104, USA
*Correspondence: herlynm@wistar.org
DOI 10.1016/j.ccr.2008.02.015

Tumor stem or initiating cells have been proposed to exist for melanoma. Stem-like cells have been propagated from melanoma cell lines and specimens. Additionally, classical stem cell markers, including ABCG2 and CD133, have been identified in clinical melanomas. However, definitive markers for the purification and further characterization of melanoma-initiating cells remained elusive. Recently, Schatton et al. provided solid evidence that the doxorubicin-resistant ATP-binding cassette transporter ABCB5 marks primitive cells capable of recapitulating melanomas in xenotransplantation models. The identification of melanoma-initiating cells has far-reaching implications, as new therapeutic strategies can be envisioned that specifically target these cells.

A cancer stem cell hierarchy has been suggested to exist for melanomas in which primitive self-renewing melanoma cells, capable of initiating tumorigenesis, give rise to rapidly proliferating, more differentiated, and tumorigenically exhausted cells that constitute the bulk tumor population. Stem-like cells have been propagated from both cultured melanoma cells and fresh clinical specimens as nonadherent spheres in stem cell-supportive media, similar to mammo- and neurospheres, that could self-renew, differentiate into various mesenchymal lineages, and initiate tumors in xenotransplantation models with small cell number (Fang et al., 2005). Furthermore, stem cell properties, including side population, and stem cell makers, including ABCG2, CD133, and nestin, have been identified in melanomas (Dou et al., 2007; Grichnik et al., 2006; Klein et al., 2007; Monzani et al., 2007). Despite this suggestive evidence, in depth in

vivo analysis of melanoma stem cells remained deficient. Markers that could specifically identify and purify putative melanoma-initiating cells for further characterization remained obscure. Recently, by applying in vivo methodologies previous utilized to identify and characterize cancer stem cells from other malignancies, Schatton et al. have demonstrated that ABCB5+ melanoma cells are essential for melanoma induction and proliferation in xenotransplantation models (Schatton et al., 2008).

Originally characterized as a regulator of cell-cell fusion of cultured progenitor melanocytes by this same group (Frank et al., 2003), ABCB5 was further shown to be a major efflux mediator of doxorubicin in melanomas (Frank et al., 2005). Moreover, ABCB5 was preferentially expressed on a distinct subset of chemoresistant CD133+ tumor cells (Frank et al., 2005), indicating that this ABC transporter may mark more primitive melanoma cells. Upon assessment of ABCB5 expression through tissue microarrays encompassing the various stages of melanoma progression, significantly elevated levels of ABCB5+ cells were observed in primary and metastatic melanomas compared to benign nevi, suggesting that ABCB5 may be a marker of melanoma progression. Further analysis of ABCB5+ melanoma cells from freshly resected metastatic melanomas demonstrated a consistently identifiable subpopulation of ABCB5-expressing cells ranging from approximately 2% to 20% of the total tumor population. Expression of ABCB5 also strongly overlapped with determinants of more primitive cells, including nestin, TIE1, CD144, and BMPR1A, implying that ABCB5+ melanoma cells possess features of stem cells. Upon xenotransplantation to a NOD/SCID mouse model, only ABCB5+ cells from either clinical melanomas or cultured melanoma cells consistently initiated tumors that were serially transplantable and capable of re-establishing the original parent tumor heterogeneity. Despite serial passage in mice, ABCB5 percentages remained similar to the original parent tumor and maintained coexpression of primitive cell markers, CD144 and TIE1.

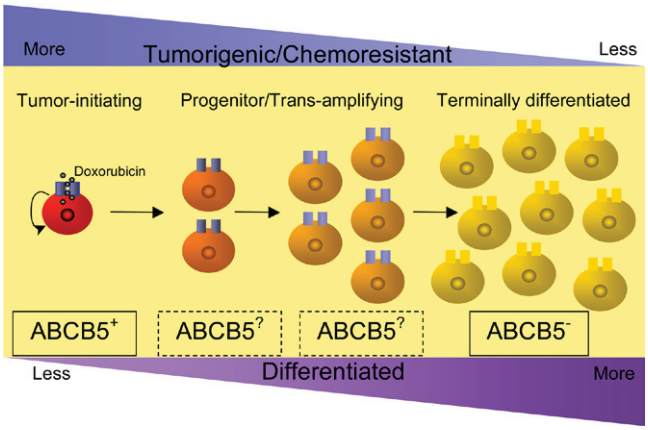

Figure 1. ABCB5 Expression in a Melanoma Stem Cell Hierarchy
In the cancer stem cell hierarchy of melanoma, ABCB5+ melanoma-initiating cells give rise to more committed progenitor/transamplifying cells that may or may not express ABCB5, depending on the stage of differentiation, while terminally differentiated cells lack expression of ABCB5. Tumorigenic potential is lost as these cells proceed toward differentiation as well as chemoresistance due to the downmodulation of ABCB5, a doxorubicin efflux transporter.

Through clever in vivo lineage tracking of differentially fluorochome-conjugated cultured melanoma cells, co-xenografted ABCB5+ and ABCB5− subpopulations were assessed for their relative contribution to tumor growth, self-renewal, and differentiation. Xenotransplantation of ABCB5+/DsRed and ABCB5−/EYFP cells to NOD/SCID mice reconstituted at a relative abundance of 1:5, respectively, resulted in a dramatic increase in the frequency of DsRed+ tumor cells of ABCB5+ origin, up to 50% of the total tumor population by the end of 6 weeks. These findings establish greater tumorigenicity of ABCB5+ subsets in a competitive tumor development model. Further analysis demonstrated that the majority of ABCB5+ cells were of DsRed phenotype, with insignificant numbers of double-positive cells, indicating that ABCB5+ cells arose exclusively from ABCB5+ cells, thus confirming the self-renewal capacity of this subset. Additionally, ABCB5− isolates exhibited DsRed positivity, demonstrating that ABCB5+ cells possess the capacity to differentiate and give rise to ABCB5− tumor cells. Meanwhile, ABCB5−/YEFP cells gave rise exclusively to ABCB5− progeny, albeit at lower frequencies, suggesting that, although ABCB5− cells lacked the capacity to self-renew, they still retained the capacity to undergo a limited number of replications prior to terminal differentiation. Moreover, ABCB5− cells could not regenerate the ABCB5+ subset, suggesting that these cells lacked the capacity to self-renew. These findings confirm the existence of a tumor hierarchy in which ABCB5+ cells self-renew and give rise to more differentiated ABCB5− progeny.

To determine whether selective elimination of ABCB5+ cells, present among an unsegregated population of melanoma cells, could inhibit tumor formation, a monoclonal anti-ABCB5 specific antibody regimen was administered. Use of a nude mouse model, as opposed to a NOD/SCID mouse model, was ideal for this type of assessment, as nude mice are capable of IgG1-triggered immune effector responses, as well as antibody-dependent cell-mediated cytotoxicity. Regimented administration of anti-ABCB5 treatment prior to and following inoculation of tumorigenic cells significantly inhibited initial tumor formation and stunted the growth of established tumor cells, compared to control antibody treatment, which did not effect tumor formation. Antibody-dependent cell-mediated cytotoxicity of ABCB5+ melanoma cells was determined to be the mechanism of inhibition. In the inoculated mice that established tumors despite anti-ABCB5 administration, these tumors still contained ABCB5+ cells, suggesting that antibody treatment could not fully eradicate these cells once established. Notably however, among the mice treated with anti-ABCB5 that failed to develop tumors, these mice remained tumor free for more than 8 months following antibody treatment withdrawal, indicating that inhibition of melanoma-initiating cells can be potently effective long after treatment has ceased. Although these data strongly indicate that ABCB5+ cells are essential for tumor induction, many clinical melanomas are identified subsequent to melanoma establishment.

Thus, to target melanoma-initiating cells in a more realistic scenario, anti-ABCB5 treatment was undertaken on established melanoma xenografts. In vivo anti-ABCB5 administration following tumor cell inoculation considerably blocked tumor growth as compared to antibody control or untreated mice. Immunohistochemical analysis revealed foci of ABCB5 expression with macrophage infiltration corresponding to in vivo bound anti-ABCB5 monoclonal antibody that frequently bordered areas of necrosis. However, whether these tumors regain growth potential once antibody administration has ceased remains unknown.

Although ABCB5+ melanoma cells were enriched in tumor-initiating cells, at least 10^5 ABCB5+ cells were required for efficient tumor induction, suggesting that not all ABCB5+ cells are equally tumorigenic. ABCB5+ melanoma cells may encompass self-renewing tumor-initiating cells in addition to less primitive ABCB5+ progenitor cells, which may or may not be capable of self-renewal, but further give rise to ABCB5− terminally differentiated cells. The data by Schatton et al. suggest that melanomas exist in a hierarchy in which ABCB5+ cells are the melanoma-initiating cells that give rise to progressively differentiated cells that lose ABCB5 expression as they reach terminal differentiation (Figure 1). However, it is not clear at which stage of differentiation ABCB5 is lost, leaving an alternative hypothesis in mind in which the melanoma-initiating cells could originate from a more committed ABCB5+ cell that has acquired mutations granting self-renewal capacity. Thus, ABCB5 in combination with other markers may be required to obtain a pure population of tumorigenic cells that also could distinguish among the various subsets within a melanoma hierarchy.

The expression of this chemoresistant efflux transporter on melanoma-initiating cells corroborates the inherent resistance of melanomas to many chemotherapeutic interventions. Whether ABCB5 is the sole efflux transporter expressed by primitive melanoma cells or in combination with other ABC efflux transporters (La Porta, 2007) remains to be determined. Preferential expression of this transporter in this small population of primitive cells in melanoma suggests that these cells have inherent death-defying mechanisms that make their eradication exceedingly challenging with current therapeutics and may be the root cause of recurrences. However, the definitive identification of melanoma-initiating cells strongly indicates the need for new therapeutic strategies that take these cells into account. One potential strategy, as indicated by Schatton et al., is antibody-mediated immune destruction. Targeting this small population of self-renewing cells as well the bulk non-self-renewing tumor cells will in all likelihood be the most effective approach.

REFERENCES

Dou, J., Pan, M., Wen, P., Li, Y., Tang, Q., Chu, L., Zhao, F., Jiang, C., Hu, W., Hu, K., and Gu, N. (2007). Cell Mol. Immunol. *4*, 467–472.

Fang, D., Nguyen, T.K., Leishear, K., Finko, R., Kulp, A.N., Hotz, S., Van Belle, P.A., Xu, X., Elder, D.E., and Herlyn, M. (2005). Cancer Res. *65*, 9328–9337.

Frank, N.Y., Pendse, S.S., Lapchak, P.H., Margaryan, A., Shlain, D., Doeing, C., Sayegh, M.H., and Frank, M.H. (2003). J. Biol. Chem. *278*, 47156–47165.

Frank, N.Y., Margaryan, A., Huang, Y., Schatton, T., Waaga-Gasser, A.M., Gasser, M., Sayegh, M.H., Sadee, W., and Frank, M.H. (2005). Cancer Res. *65*, 4320–4333.

Grichnik, J.M., Burch, J.A., Schulteis, R.D., Shan, S., Liu, J., Darrow, T.L., Vervaert, C.E., and Seigler, H.F. (2006). J. Invest. Dermatol. *126*, 142–153.

Klein, W.M., Wu, B.P., Zhao, S., Wu, H., Klein-Szanto, A.J., and Tahan, S.R. (2007). Mod. Pathol. *20*, 102–107.

La Porta, C.A. (2007). Curr. Med. Chem. *14*, 387–391.

Monzani, E., Facchetti, F., Galmozzi, E., Corsini, E., Benetti, A., Cavazzin, C., Gritti, A., Piccinini, A., Porro, D., Santinami, M., et al. (2007). Eur. J. Cancer *43*, 935–946.

Schatton, T., Murphy, G.F., Frank, N.Y., Yamaura, K., Waaga-Gasser, A.M., Gasser, M., Zhan, Q., Jordan, S., Duncan, L.M., Weishaupt, C., et al. (2008). Nature *451*, 345–349.