Commentary: Viewing malignant melanoma cells as macrophage-tumor hybrids.

John M. Pawelek, PhD, Research Affiliate, Dept of Dermatology and the Yale Cancer Center, Yale School of Medicine, New Haven, CT, USA (john.pawelek@yale.edu)

The Pan American Society for Pigment Cell Research
(Also presented to The Society for Melanoma Research)
March 1, 2006

Introduction. Here I suggest that the defining characteristics of malignant melanoma are a consequence of fusion of melanoma cells with macrophages (1,2). While there is genetic proof for melanoma hybrids in mice, there is as yet no genetic 'smoking gun' in human melanoma. Thus, the arguments below for hybrids in human melanoma are based on phenotypic similarities to documented melanoma hybrids in mice and *in vitro* models.

Hybridization as an evolutionary mechanism. In bacteria, an average 14% of the genes were acquired through horizontal gene transfer from other bacteria (3). In eukaryotes, mitochondria and chloroplasts arose through acquisition of ancient prokaryotic genomes (4,5). In mammalian tissues, bone marrow-derived cells contribute to a wide variety of normal tissues such as liver, brain, and heart. This is due at least in part to cell fusion with monocyte/macrophage-lineage cells. Hybridization represents a mechanism for acquiring many new genes at once. Thus hybridization has enormous power for rapid selective advantage.

Fusion hybrids in cancer. Given that genomic hybridization is widely prevalent in biology, why not in cancer? In fact there are numerous reports of hybridization in cancer. Many implicate macrophages or other bone marrow-derived cells as fusion partners. And many further implicate hybridization as the cause of metastasis (1,2). How extensive is this phenomenon? It may be telling that of the more than 25 reports of tumor hybridization *in vivo*, none has reported a failure to detect hybrids. This includes two recent reports of bone marrow-tumor cell hybrids in human renal cell carcinoma (6,7).

There is a vast repertoire of molecules and traits shared by macrophages and melanoma cells. Some are associated with angiogenesis, matrix alterations, motility, chemotaxis and immune signaling pathways. Others involve expression of macrophage-like phenotypes or are immunomarkers used in the identification of macrophages. Macrophage fusion could explain the aneuploidy, plasticity, and heterogeneity of malignant melanoma and it could also account for epidermal-mesenchymal transition in tumor progression since macrophages are of mesodermal origin (1,2). Below is a brief consideration of phenotypic features of experimental macrophage-melanoma fusion hybrids that are also associated with human malignant melanoma: hyperpigmentation, coarse melanin, and aberrant glycosylation in the form of β1,6-branched oligosaccharides.

Spontaneous *in vivo* hybrids in melanoma are hypermelanotic, contain coarse melanin and exhibit β1,6-branched oligosaccharides. Three reports describe

melanoma-host hybrids in mice: one of B16 melanoma cells (8) and two of Cloudman S91 melanoma cells (9,10). In all three cases, the hybrids were hypermelanotic, showed increased dendricity and were hyperploid compared to the parental melanoma cells. Perhaps most important, these lines showed markedly higher tumorigenicity (8) and metastatic potential (9-11). We tested two of these hybrids and found that they produced 'coarse melanin', phagolysosomal-like vesicles with multiple melanosomes (below). Likewise, when mouse or human macrophages were experimentally fused with Cloudman S91 melanoma cells *in vitro*, more than half of the 75 individual hybrids were of increased metastatic potential and most of these were hypermelanotic and produced coarse melanin (11,12). Highly metastatic, hypermelanotic macrophage-melanoma hybrid 48 is shown below (Fig 1, right) compared to the non-pigmented parental Cloudman S91 melanoma cells (Fig 1, left) (from 13).

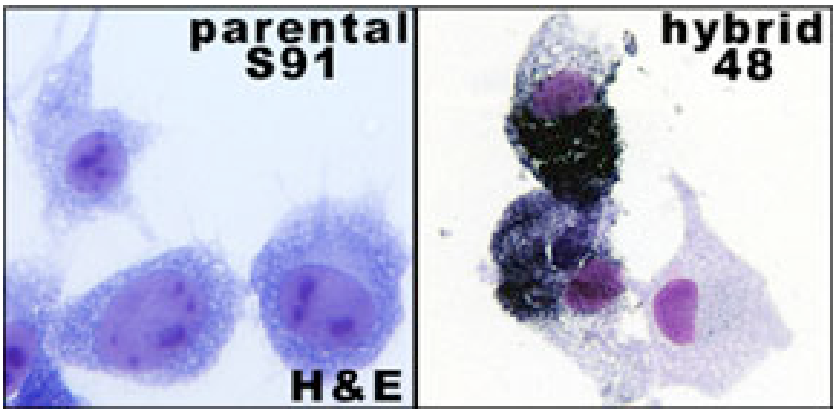

Figure 1. Left: Non-pigmented Cloudman S91 melanoma, the parental fusion partner. Right: Highly melanized melanoma-macrophage hybrid 48 (13).

EM views of coarse melanin are shown below in hybrid 48 and in B16F10 cells (Fig 2).

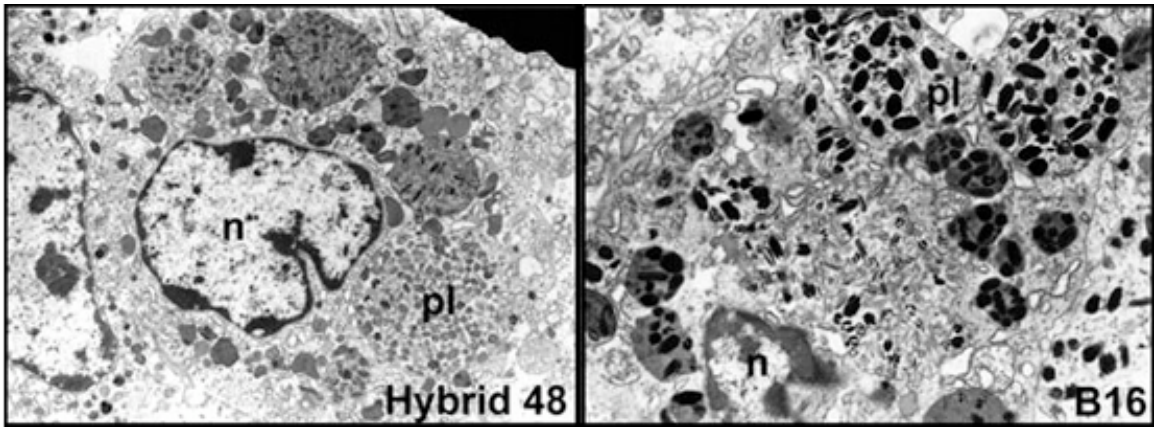

Figure 2. Left: EM of melanoma-macrophage hybrid 48. Right: A B16F10 mouse melanoma cell. Each cell line produced coarse melanin: phagolysosome-like structures containing multiple melanosomes. (n, nucleus; pl, phagolysosome).

The hypermelanotic phenotype was puzzling. Why should fusion of a non-pigmented macrophage with a weakly-pigmented melanoma cell yield highly pigmented hybrids? The short answer is aberrant glycosylation in the form of β1,6-branched oligosaccharides.

Analyses of melanosomal proteins tyrosinase, TYRP-1, TYRP-2, and LAMP-1 in macrophage-melanoma hybrids showed that they were heavily glycosylated compared to parental melanoma cells. That LAMP-1 was one of these proteins provided the first indication that β1,6-branched oligosaccharides might be involved (12). LAMP-1 is one of the most heavily glycosylated of all proteins and is the chief substrate for GnT-V, a glycosyltransferase that is rate-limiting in the formation of β1,6-branched oligosaccharides. GnT-V activates several pathways in metastastic progression. High GnT-V expression is a macrophage trait and it thus seemed likely that GnT-V might be elevated in macrophage-melanoma hybrids due to genetic input from the parental macrophage. Indeed, GnT-V and β1,6-branched oligosaccharides were elevated in the high metastatic hybrids (14). Use of glycosylation inhibitors and selective GnT-V inactivation in hybrids revealed that GnT-V expression was the underlying cause for both chemotactic motility and hyperpigmentation (12, Chakraborty et al, submitted).

It is possible that GnT-V itself induced formation of coarse vesicles. GnT-V transfection into mink lung cells induced production of LPHA-positive multilamellar vesicles and the process was dependent on autophagy (15,16). Likewise, in macrophage-melanoma hybrids with high GnT-V expression, β1,6-branched oligosaccharides co-localized with coarse melanin vesicles (detected with the lectin LPHA) (Fig 3, upper). Similarly, human Skmel-2 melanoma cells expressed LPHA-positive coarse melanin (Fig 3, lower).

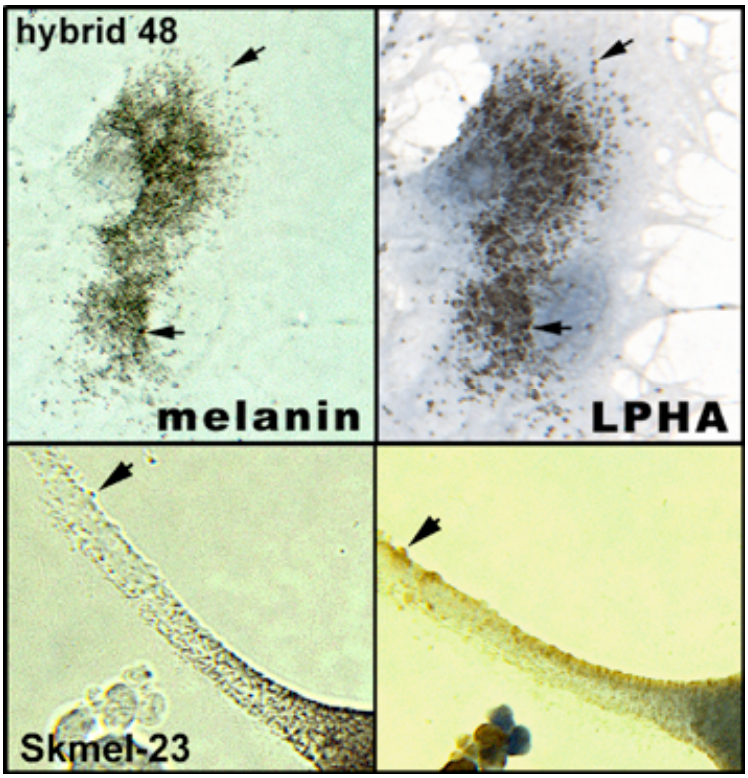

Figure 3. β1,6-branched oligosaccharides co-localized to coarse melanin. Upper Left: Coarse melanin in macrophage-Cloudman melanoma hybrid 48. Right: The same cell bleached and stained for β1,6-branched oligosaccharides with the lectin LPHA. Lower left: Coarse melanin in Skmel-23 human melanoma cells. Lower right: the same cell stained for β1,6-branched oligosaccharides. Arrows denote co-localization (13).

Histological correlates in human melanoma. Prompted by the above findings, we looked for similar phenotypes in pathology specimens of human melanoma. A major diagnostic feature of primary CMM is heterogeneous pigmentation with prominent hyper- and/or hypomelanotic regions. We found that the hypermelanotic regions of CMM consist of coarse melanin-producing melanoma cells and melanophages, both of which strongly expressed β1,6-branched oligosaccharides (Fig 4) (16). A similar phenotype was seen in human melanoma cell lines, e.g. the SKmel-23 line (Fig 3, lower) (13). These vesicles have been long been observed by dermatopathologists and were noted to be associated with invasive melanoma (17).

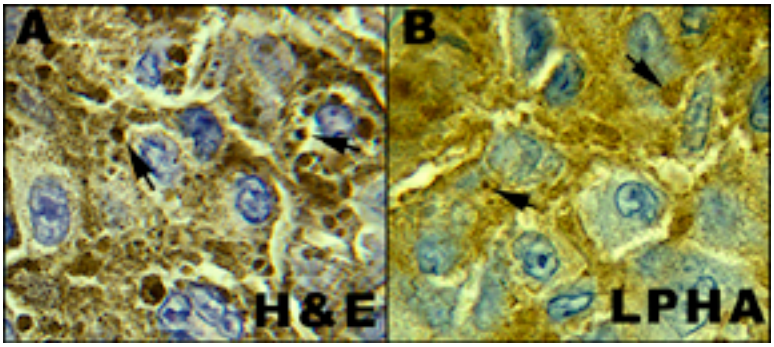

Figure 4. Human cutaneous malignant melanoma *in situ*. Left: Coarse melanin in an H&E-stained section (arrows). Right: A sequential section bleached to decolorize melanin and stained for β1,6-branched oligosaccharides with LPHA (from Handerson and Pawelek, 2003 (16)).

Expression of β1,6-branched oligosaccharides is widespread in CMM. When melanoma tissue microarrays with more than 500 primary and metastatic tumors were stained with LPHA, 80-90% of the tumors scored positive (Handerson et al, in preparation). The phenotype of β1,6-branched oligosaccharides and coarse vesicles was also seen in some nevi, however melanocytes peripheral to the tumors did not express β1,6-branched oligosaccharides or coarse melanin (16).

In addition to β1,6-branched oligosaccharides, numerous other melanoma traits are expressed by macrophages (1,2). In an example of current interest, focal adhesion kinase (FAK) which is expressed by macrophages (18) was recently found to be associated with plasticity, vasculogenic mimicry and aggressiveness in human melanoma (19).

Is the B16 melanoma a macrophage hybrid? From the great number of citations, the B16 melanoma is surely the most widely studied of all the melanoma models. In light of the discussion herein, it is important to note that, in retrospect, the *in vivo* generation of B16F10 melanoma cells was favorable for host-tumor hybrid formation (20). B16 tumor cells were cycled repeatedly from culture, to mice, to culture. Each time, lung nodules were dissociated, placed in culture and "colonies with melanin granules" (20) were selected for further cycling in mice. As mentioned above, there are three reports that *in vivo* cycling of melanoma cells produced host-melanoma hybrids (8-10). B16F10 melanoma cells expressed the same phenotype as these hybrids: high metastatic potential, hyperpigmentation, high expression of GnT-V and β1,6-branched oligosaccharides, and coarse melanin (Fig 2, right). Thus, the possibility is raised that B16F10 melanoma cells

are macrophage-tumor hybrids. However, since B16F10 melanoma cells were generated in syngeneic (C57B6) mice, it has not been possible to determine this on a genetic basis.

Genetic implications of hybridization. In considering gene expression in hybrids, it would seem that the phenotype of macrophage-tumor fusion hybrids would be defined by the sum of gene expression from cells of different developmental lineages--each fusion partner being imprinted for gene expression from its own lineage. This could explain the co-expression of macrophage and melanocyte traits in CMM. Metastatic cells would arise when the migratory abilities of myeloid cells and the uncontrolled proliferation of tumor cells were co-expressed in hybrids. Aneuploidy and heterogeneity would occur through variations in the hybrid genome that would likely differ between individual hybrids.

Is the tumor environment conducive to hybrid formation? The melanoma tumor environment is rich in melanophages (macrophages that injest melanin). Macrophages actively phagocytose apoptotic cells. Oncogenes in apoptotic cells can be horizontally transferred via phagocytosis where they confer a tumorigenic phenotype (21). Macrophages also express inherent mechanisms for cell-cell fusion as in the formation of osteoclasts and multinucleated giant cells. Below are pathology specimens of human melanomas stained for melanophages (arrows) by the S100/azure blue technique. Melanophages are seen in close association with melanoma cells with long segments of plasma membranes of the two cell types in apposition to one another (Fig 1a and b), or with melanophages engulfing melanoma cells (Fig 1c). Physical contact such as this would be a prerequisite for fusion and macrophage-tumor hybrid formation. Thus, it would seem that the melanoma tumor environment is conducive to macrophage-melanoma fusion.

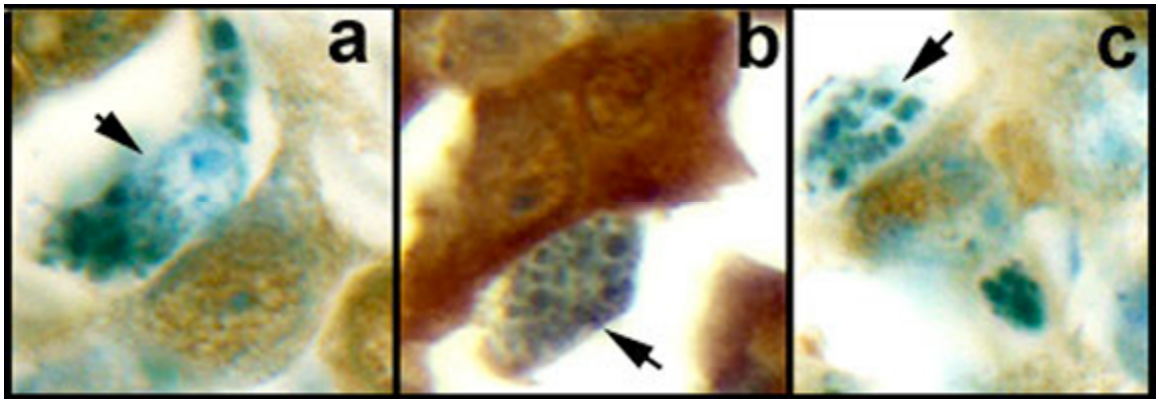

Figure 5. Melanophages (arrows) staining blue-green with azure blue in close association with melanoma cells staining brown with the S100-immunoperoxidase reaction. Images are from archival pathology cases of human CMM (Handerson and Pawelek, unpublished).

Conclusions. I therefore propose that human malignant melanoma may be a disease of tumor-macrophage hybridization. Although there certainly could be other mechanisms, they would need to explain the remarkable similarities between hyperpigmented human melanoma cells and melanoma-macrophage hybrids. But genetic proof for hybrids in human melanoma is still lacking. Cases in which patients develop melanoma following a bone marrow transplant could provide such evidence and we would very much appreciate

being alerted to any such cases. I hope that these comments have helped to better understand the possibility of macrophage-melanoma fusion in malignant melanoma.

References

1. Pawelek J. Tumor cell fusion as a source of myeloid traits in cancer. The Lancet Onclogy 6: 988-993, 2005. (accompanying commentary: Friedl P. Cell fusion: new mechanisms of plasticity in cancer? Lancet Oncol. 2005;12:916-918.)

2. Pawelek J, Chakraborty A, Lazova, R, Yilmaz Y, Cooper D, Brash D, and Handerson T. Co-opting macrophage traits in cancer progression: a consequence of tumor cell fusion? In: Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis. Contrib. Microbiol. Basel, Karger, 2006, vol 13, pp 138-155.

3. Nakamura Y, Itoh T, Matsuda H, Gojobori T. Biased biological functions of horizontally transferred genes in prokaryotic genomes. Nat Genet. 2004;36:760-6

4. Margulis L. 1970.*Origins of Eukaryotic Cells*. New Haven, CT. Yale Univ Press. 349pp.

5. Lang BF, Gray MW, Burger G. Mitochondrial genome evolution and the origin of eukaryotes. Annu Rev Genet. 1999;33:351-97. Review.

6. Chakraborty, A., Lazova, R., Davies, S., Bäckvall, H., Ponten, F., Brash, D., and Pawelek, J. Donor DNA in a renal cell carcinoma metastasis from a bone marrow transplant recipient. Bone Marrow Transplantation 34: 183-186, 2004.

7. Yilmaz, Y., Lazova, R., Qumsiyeh, M., Cooper, D., and Pawelek, J. Donor Y chromosome in renal carcinoma cells of a female BMT recipient. Bone Marrow Transplant 35:1021-1024, 2005.

8. Hu F and Pasztor LM, 1975, *In vivo* hybridization of cultured melanoma cells and isogenic normal mouse cells. Differentiation, 4, 93-97

9. Halaban R, Nordlund J, Francke U, Moellmann G and Eisenstadt JM, 1980, Supermelanotic hybrids derived from mouse melanomas and normal mouse cells. Somatic Cell Genetics, 6, 29-44.

10. Chakraborty A. K., Sodi S., Rachkovsky M., Kolesnikova N., Platt J. T., Bolognia J. L., Pawelek J. M. A spontaneous murine melanoma lung metastasis comprised of host x tumor hybrids. Cancer Res., 60*:* 2512-2519, 2000.

11. Rachkovsky MS, Sodi S, Chakraborty A, Avissar Y, Bolognia J, Madison J, Bermudes D, and Pawelek J. Enhanced metastatic potential of melanoma x macrophage fusion hybrids. Clin Exp Metastasis 16: 299-312, 1998.

12. Pawelek, J. M., Chakraborty, A. K., Rachkovsky, M. L., Orlow, S. J., Bolognia, J. L., and Sodi, S. A. Altered N-glycosylation in macrophage x melanoma fusion hybrids. Cell. Mol. Biol., 45: 1011-1027, 2000.

13. Rupani, R., Handerson, T., and Pawelek, J. Co-localization of β1,6-branched oligosaccharides and coarse melanin in macrophage-melanoma fusion hybrids and human melanoma cells in vitro. Pigment Cell Res. 17: 281-288, 2004.

14. Chakraborty, A,K., Pawelek, J.M, Ikeda, Y., Miyoshi, E., Kolesnikova, N., Funasaka, Y., Ichihashi, M., and Tanaguchi, N. Macrophage fusion up-regulates N-acetyl-glucosaminyltransferase V, β1,6 branching, and metastasis in Cloudman S91 mouse melanoma cells. Cell Growth and Diff. 12: 623-630, 2001.

15. Hariri M., Millane G., Guimond M., Guay G., Dennis J.W., Nabi I.R. Biogenesis of multilamellar bodies via autophagy. Mol. Biol. Cell 11:255-268, 2000.

16. Handerson, T., and Pawelek, J. β1,6-branched oligosaccharides and coarse vesicles: A common and pervasive phenotype in melanoma and other human cancers. Cancer Res. 63: 5363-5369, 2003.

17. Clark WH, Mastangelo MJ, Ainsworth AM, Berd D, Bellet RE, Bernardino EA. Current concepts of the biology of human cutaneous malignant melanoma. Adv Cancer Res. 24: 267-338, 1977.

18. Rovida E, Lugli B, Barbetti V, Giuntoli S, Olivotto M, Dello Sbarba P. Focal adhesion kinase is redistributed to focal complexes and mediates cell spreading in macrophages in response to M-CSF. Biol Chem. 2005;386:919-929.

19. Hess AR, Postovit LM, Margaryan NV, Seftor EA, Schneider GB, Seftor RE, Nickoloff BJ, Hendrix MJ. Focal adhesion kinase promotes the aggressive melanoma phenotype. Cancer Res. 2005;65:9851-9860.

20. Fidler IJ.Selection of successive tumour lines for metastasis. Nat New Biol. 1973; 242:148-149.

21. Holmgren L, Bergsmedh A, Spetz AL: Horizontal transfer of DNA by the uptake of apoptotic bodies. Vox Sang 2002;83 Suppl 1:305-306.