to treat organ fibrosis mainly target fibroblasts, this paper certainly offers new therapeutic perspectives by identifying other non-hematopoietic cells, such as, for instance, epithelial cells, as potential targets.

REFERENCES

Adriaens, C., Standaert, L., Barra, J., Latil, M., Verfaillie, A., Kalev, P., Boeckx, B., Wijnhoven, P.W., Radaelli, E., Vermi, W., et al. (2016). p53 induces formation of NEAT1 lncRNA-containing paraspeckles that modulate replication stress response and chemosensitivity. Nat. Med. *22*, 861–868.

Fukushima, K., Satoh, T., Sugihara, F., Sato, Y., Okamoto, T., Mitsui, Y., Yoshio, S., Li, S., Nojima, S., Motooka, D., et al. (2020). Dysregulated Expression of the Nuclear Exosome Targeting Complex Component Rbm7 in Non-hematopoietic Cells Licenses the Development of Fibrosis. Immunity *52*, this issue, 542–556.

Gibbons, M.A., MacKinnon, A.C., Ramachandran, P., Dhaliwal, K., Duffin, R., Phythian-Adams, A.T., van Rooijen, N., Haslett, C., Howie, S.E., Simpson, A.J., et al. (2011). Ly6Chi monocytes direct alternatively activated profibrotic macrophage regulation of lung fibrosis. Am. J. Respir. Crit. Care Med. *184*, 569–581.

Griffiths, K., Habiel, D.M., Jaffar, J., Binder, U., Darby, W.G., Hosking, C.G., Skerra, A., Westall, G.P., Hogaboam, C.M., and Foley, M. (2018). Anti-fibrotic Effects of CXCR4-Targeting i-body AD-114 in Preclinical Models of Pulmonary Fibrosis. Sci. Rep. *8*, 3212.

Kim, K.K., Dotson, M.R., Agarwal, M., Yang, J., Bradley, P.B., Subbotina, N., Osterholzer, J.J., and Sisson, T.H. (2018). Efferocytosis of apoptotic alveolar epithelial cells is sufficient to initiate lung fibrosis. Cell Death Dis. *9*, 1056.

Lee, J.M., Yoshida, M., Kim, M.S., Lee, J.H., Baek, A.R., Jang, A.S., Kim, D.J., Minagawa, S., Chin, S.S., Park, C.S., et al. (2018). Involvement of Alveolar Epithelial Cell Necroptosis in Idiopathic Pulmonary Fibrosis Pathogenesis. Am. J. Respir. Cell Mol. Biol. *59*, 215–224.

Misharin, A.V., Morales-Nebreda, L., Reyfman, P.A., Cuda, C.M., Walter, J.M., McQuattie-Pimentel, A.C., Chen, C.I., Anekalla, K.R., Joshi, N., Williams, K.J.N., et al. (2017). Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span. J. Exp. Med. *214*, 2387–2404.

Reyfman, P.A., Walter, J.M., Joshi, N., Anekalla, K.R., McQuattie-Pimentel, A.C., Chiu, S., Fernandez, R., Akbarpour, M., Chen, C.I., Ren, Z., et al. (2019). Single-Cell Transcriptomic Analysis of Human Lung Provides Insights into the Pathobiology of Pulmonary Fibrosis. Am. J. Respir. Crit. Care Med. *199*, 1517–1536.

Satoh, T., Nakagawa, K., Sugihara, F., Kuwahara, R., Ashihara, M., Yamane, F., Minowa, Y., Fukushima, K., Ebina, I., Yoshioka, Y., et al. (2017). Identification of an atypical monocyte and committed progenitor involved in fibrosis. Nature *541*, 96–101.

Wynn, T.A., and Vannella, K.M. (2016). Macrophages in Tissue Repair, Regeneration, and Fibrosis. Immunity *44*, 450–462.

An Inbred Ecosystem that Supports Medulloblastoma

Ana de Pablos-Aragoneses[1] and Manuel Valiente[1,*]
[1]Brain Metastasis Group, Spanish National Cancer Research Center, Madrid, Spain
*Correspondence: mvaliente@cnio.es
https://doi.org/10.1016/j.immuni.2020.02.010

In a recent issue of *Cell*, Yao et al. use a unique genetic strategy to study sonic hedgehog-medulloblastoma. Their results reveal a complex network in the tumor microenvironment involving the *trans*-differentiation of cancer cells into astrocytes to fuel tumor growth.

Driving your car to work, playing ball with the kids, or even walking to the kitchen to have breakfast every morning—the cerebellum coordinates of all these actions. This part of the brain is a vital player in fine movement and coordination, but it is also the origin of the most common solid cancer in children: medulloblastoma (MB). Out of the four MB subtypes (Hovestadt et al., 2020), sonic hedgehog (SHH)-MB is a genetically simple brain tumor that has been studied in great detail. Building on this well-established brain tumor subtype, the authors have analyzed the surrounding microenvironment to made a striking discovery: the cell or origin of this tumor is not only able to generate cancer cells but also its own microenvironment, which corrupts resident brain cells to foster tumor growth (Figure 1).

During normal cerebellar development, granule neural progenitors (GNPs), located in the outermost layer of the cerebellum, named external granule layer (EGL), undergo massive proliferation regulated by the SHH receptor patched-1. However, a constitutive activation of SHH-dependent signaling in the GNPs could result in MB (Hovestadt et al., 2020). When SHH is not present, patched-1 prevents cells from growing and dividing by repressing the G protein-coupled receptor smoothened (SMO). Based on its role in preventing cells from proliferating in an uncontrolled way, *PTCH1* is one of the best-known tumor suppressor genes in SHH-MB. Germline mutations in *PTCH1* can result in development of MB (Hovestadt et al., 2020), and mice bearing heterozygous mutations of *Ptch1* have been shown to faithfully model the human disease. Moreover, it has been shown that *p53* loss can enhance the SHH-driven tumorigenesis and the incidence of MB in a *Ptch1*$^{+/-}$ background mice.

Available SMO inhibitor vismodegib induced positive therapeutic responses in *PTCH1* mutated SHH-MB (Hovestadt et al., 2020). However, bone morbidities in small children derived from its use have limited this therapeutic option (Hovestadt et al., 2020). Consequently, alternative approaches are highly needed. The authors argued that exploring the tumor microenvironment (TME) might

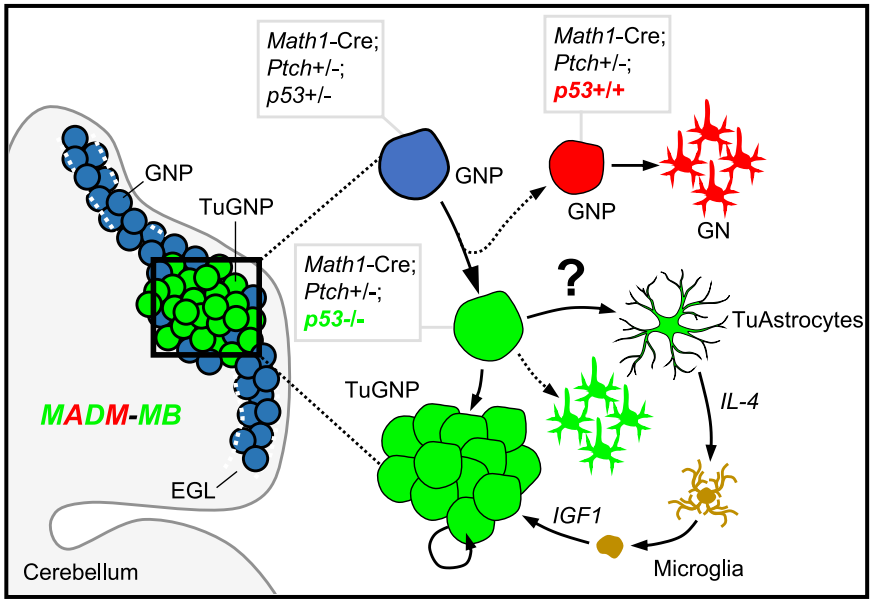

Figure 1. MADM Uncovers Microenvironment Crosstalk in SHH-MB
Granule neural progenitors (GPNs) located in the external granule layer (EGL) of the cerebellum are the cell of origin of SHH-MB. The technique MADMs allows the authors to trace mutant cells that are differentially labeled in green in contrast to red cells. GFP+ mutant GNPs form tumors (TuGNPs) and *trans*-differentiate into tumor-derived astrocytes (TuAstrocytes). This process establishes a novel network in the TME, since TuAstrocytes activates microglia in an IL-4-dependent manner that leads to the production of insulin growth factor (IGF-1), which is the final inducer of TuGNP proliferation. GN, granule neurons.

uncover novel vulnerabilities of this childhood tumor.

With the objective of obtaining the highest temporospatial resolution of TME, Yao et al. (2020) have applied an elegant tool termed Mosaic Analysis with Double Markers system (MADMs). This method allows for the generation of genetic mosaicism in mice in which sibling mutant and wild-type cells are labeled with different fluorescent markers. MADMs makes use of Cre-loxP-dependent interchromosomal mitotic recombination to generate uniquely labeled homozygous mutant cells in an otherwise heterozygous background in mice (Zong et al., 2005). MADMs represents a tool to model different types of cancers (Muzumdar et al., 2016), as it allows to generate conditional knockouts of tumor suppressor genes creating sparse loss-of-heterozygosity events to follow tumor progression *in vivo*. In the context of SHH-MB, the rarity of p53 null cells, resulting from the low efficiency of recombination between two homologous chromosomes, allows to mimic the clonal origin of cancer in human patients and the possibility to track the different subpopulations throughout the entire tumorigenic process.

In their model, *Math1*-driven Cre, which is specifically expressed in GNPs, together with MADMs generates red fluorescent protein (RFP)$^{+}p53^{+/+}$ and green fluorescent protein (GFP)$^{+}p53^{-/-}$ GNPs in a *Ptch1*$^{+/-}$ mouse. This strategy allows the authors to track the tumor originating cells, those labeled in green ($p53^{-/-}$, *Ptch1*$^{+/-}$, GFP^{+}).

The simple genetic makeup of this tumor and the fast growth rates in the brain derived from SHH pathway activation are in sharp contrast to the well-known poor ability of these cancer cells to grow *in vitro*. This simple fact suggests that additional non-cancer components of the TME might play a critical role in SHH-MB progression. Previous research efforts have proposed that the microenvironment is key in the biology of brain tumors. Yao et al. (2020) demonstrate that the tumor and its surrounding microenvironment co-evolve, establishing new intercellular networks.

What do we do when we arrive to a new place for living? We tend to remove things that we don't like; we make friends with the neighbors; we decorate our house with things that make us feel better. In summary, we influence and modify the surroundings to make our lives easier. Cancer cells perform a similar process within the hostile brain ecosystem to make the environment better suit their needs.

The brain as a soil is unique (i.e., the extracellular matrix and many of the tissue-resident cell types—microglia, astrocytes, and neurons—are not found anywhere else); it is physically isolated by the blood-brain barrier, and homeostasis is governed by huge metabolic demands, and local defensive strategies are designed to neutralize potential threads as soon as possible to compensate for the limited regenerative potential of this organ. Cancer cells have to deal with and overcome all these aspects in order to survive and colonize.

Yao et al. (2020) report that one strategy that cancer cells use is to promote their survival is to generate their own microenvironment. As demonstrated previously, MB cells are not able to grow under *in vitro* conditions; therefore, the authors hypothesized a strong contribution from the TME, focusing on the glial compartment: astrocytes and microglia. Unexpectedly, the authors found that 100% of tumor-associated astrocytes are GFP+, indicating that they are most likely derived from tumor GNPs (TuGNPs) rather than normal astrocytes (Yao et al., 2020). To rule out technical caveats with this interpretation, the authors provide additional supporting evidence as well as develop different genetic strategies that supported their hypothesis. Among them, they showed that astrocytes have the identical chromosomal loss of *PTCH1* locus as surrounding tumor cells in both patient-derived MB samples and in the MB mouse model. This indicates that the astrocytes are likely tumor derived. Although their findings suggest this conclusion, additional work is needed to definitely demonstrate the main claim of this publication.

The authors go on to show that these tumor-derived astrocytes (TuAstrocytes) produce interleukin (IL)-4, which hijacks microglia function for the benefit of the tumor. IL-4-stimulated microglia modify their own secretome and start producing the cytokine IGF-1, which signals back to cancer cells to promote MB outgrowth. This complicated multicellular network involving cancer cells and various components of the microenvironment provides a

potential explanation to the difficulties of culturing MB cells *in vitro* by their own. Therefore, these findings indicate that the tumor is modifying its own environment by promoting the *trans*-differentiation of TuGNPs into TuAstrocytes to fuel tumor growth.

The next logical question, which was not addressed in the manuscript (Yao et al., 2020), is what would be the mechanism regulating *trans*-differentiation of cancer cells into astrocytes. *trans*-differentiation, in contrast to reprogramming, does not require full reversal of the original cell of interest into a pluripotent stem cell to give rise to another cell type. In some cases, *trans*-differentiation is a one-step process, while in others, the cells pass through one or several intermediate states. It has been discussed before that cancer stem cells can potentially *trans*-differentiate to lineages other than the one from which the tumor arose (Shekhani et al., 2013). The *trans*-differentiation process from TuGNPs is quite shocking, because GNPs normally do not generate astrocytes, and thus, the progenitors of normal astrocytes and TuAstrocytes differ both temporally and spatially.

Previous publications have partially addressed the potential origin of disease-associated astrocytes. The high functional variability of astrocytes in health (Khakh and Sofroniew, 2015) might originate from existing differences at the level of progenitor cells. Alternatively, injury has been shown to activate quiescent progenitors present in the murine adult brain that give rise to *de novo*-generated astrocytes (Benner et al., 2013). Also, the local environment where astrocytes perform their function (i.e., perivascular) (Bardehle et al., 2013) could contribute to shape different functional subtypes. Most likely, astrocytes will evolve throughout the course of brain disorders. For instance, in the context of brain metastasis, reactive astrocytes play an anti-metastatic role that limits disease progression during early stages (Valiente et al., 2014), while later on, they become strongly pro-metastatic (Priego et al., 2018). Thus, a comparative analysis of the presence of *trans*-differentiated TuAstrocytes with other brain tumors as well as a more detailed characterization of the molecular regulation of this process is highly needed.

Understanding the regulatory mechanisms of emerging tumor-associated intercellular networks are important not only to fully explain the biology of brain tumors but also to develop innovative therapies targeting not cancer cells directly, but the altered microenvironment.

ACKNOWLEDGMENTS

Research in the Brain Metastasis Group is supported by MINECO grants MINECO-Retos SAF2017-89643-R (M.V.), Bristol-Myers Squibb-Melanoma Research Alliance Young Investigator Award 2017 (498103) (M.V.), Fundación Ramón Areces (CIVP19S8163) (M.V.), Worldwide Cancer Research (19-0177) (M.V.), H2020-FETOPEN (828972) (M.V.), Clinic and Laboratory Integration Program CRI Award 2018 (54545) (M.V.), AECC Coordinated Translational Groups 2017 (GCTRA16015SEOA) (M.V.), LAB AECC 2019 (LABAE19002VALI) (M.V.), and La Caixa INPhINIT Fellowship (LCF/BQ/DI19/11730044) (A.d.P.-A.). M.V. is a Ramón y Cajal Investigator (RYC-2013-13365) and EMBO YIP (4053).

REFERENCES

Bardehle, S., Krüger, M., Buggenthin, F., Schwausch, J., Ninkovic, J., Clevers, H., Snippert, H.J., Theis, F.J., Meyer-Luehmann, M., Bechmann, I., et al. (2013). Live imaging of astrocyte responses to acute injury reveals selective juxtavascular proliferation. Nat. Neurosci. *16*, 580–586.

Benner, E.J., Luciano, D., Jo, R., Abdi, K., Paez-Gonzalez, P., Sheng, H., Warner, D.S., Liu, C., Eroglu, C., and Kuo, C.T. (2013). Protective astrogenesis from the SVZ niche after injury is controlled by Notch modulator Thbs4. Nature *497*, 369–373.

Hovestadt, V., Ayrault, O., Swartling, F.J., Robinson, G.W., Pfister, S.M., and Northcott, P.A. (2020). Medulloblastomics revisited: biological and clinical insights from thousands of patients. Nat. Rev. Cancer *20*, 42–56.

Khakh, B.S., and Sofroniew, M.V. (2015). Diversity of astrocyte functions and phenotypes in neural circuits. Nat. Neurosci. *18*, 942–952.

Muzumdar, M.D., Dorans, K.J., Chung, K.M., Robbins, R., Tammela, T., Gocheva, V., Li, C.M.-C., and Jacks, T. (2016). Clonal dynamics following p53 loss of heterozygosity in Kras-driven cancers. Nat. Commun. *7*, 12685.

Priego, N., Zhu, L., Monteiro, C., Mulders, M., Wasilewski, D., Bindeman, W., Doglio, L., Martínez, L., Martínez-Saez, E., Ramón Y Cajal, S., et al. (2018). STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis. Nat. Med. *24*, 1024–1035.

Shekhani, M.T., Jayanthy, A.-S., Maddodi, N., and Setaluri, V. (2013). Cancer stem cells and tumor transdifferentiation: implications for novel therapeutic strategies. Am. J. Stem Cells *2*, 52–61.

Valiente, M., Obenauf, A.C., Jin, X., Chen, Q., Zhang, X.H.-F., Lee, D.J., Chaft, J.E., Kris, M.G., Huse, J.T., Brogi, E., and Massagué, J. (2014). Serpins promote cancer cell survival and vascular co-option in brain metastasis. Cell *156*, 1002–1016.

Yao, M., Ventura, P.B., Jiang, Y., Rodriguez, F.J., Wang, L., Perry, J.S.A., Yang, Y., Wahl, K., Crittenden, R.B., Bennett, M.L., et al. (2020). Astrocytic *trans*-Differentiation Completes a Multicellular Paracrine Feedback Loop Required for Medulloblastoma Tumor Growth. Cell *180*, 502–520.

Zong, H., Espinosa, J.S., Su, H.H., Muzumdar, M.D., and Luo, L. (2005). Mosaic analysis with double markers in mice. Cell *121*, 479–492.