

# Notch1-MAPK Signaling Axis is Essential in CD133<sup>+</sup> Melanoma Initiating Cells

Dhiraj Kumar<sup>1</sup>, Poonam R Pandey<sup>2</sup> and Gopal C Kundu<sup>3\*</sup>

<sup>1</sup>Department of Cancer Biology, the University of Texas MD Anderson Cancer Center, Houston, Texas 77054, USA

<sup>2</sup>Laboratory of Genetics, National Institute on Aging-Intramural Research Program, National Institutes of Health, Baltimore, Maryland 21224, USA

<sup>3</sup>Laboratory of Tumor Biology, Angiogenesis and Nanomedicine Research, National Centre for Cell Science, Pune 411007, India

\*Corresponding author: Gopal C Kundu, Laboratory of Tumor Biology, Angiogenesis and Nano medicine Research, National Centre for Cell Science, Pune, India, Tel: 912025708104; E-mail: kundu@nccs.res.in

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#### Commentary

Human malignant melanoma is highly aggressive and metastatic in nature and exhibits phenotypic plasticity. Melanoma has multiple phenotypically distinct subpopulations which involved in tumor progression and markedly resistant to conventional therapy. Some of the melanoma subpopulation exhibits stem cells feature and defined as cancer stem cells (CSCs) or tumor initiating cells (TICs). Identification and characterization of CSCs in melanoma may have therapeutic implication for combating melanoma progression. In recent study, we have identified that CD133<sup>+</sup> subpopulation of melanoma potentially involved in tumor initiation, metastasis, epithelial to mesenchymal transition (EMT) and angiogenesis. Genetic and pharmacological screening revealed that functional properties of CD133<sup>+</sup> melanoma cells are regulated by Notch1-MAPK signaling axis. Andrographolide (herbal product) abrogates Notch1-MAPK pathway in CD133<sup>+</sup> cells that leads to attenuation of melanoma progression, metastasis and angiogenesis.

The study of tumor initiating cells provides a potential explanation of tumor aggressiveness, drug resistant and distant metastasis [1,2]. Several reports showed that CD133, CD20, CD271, ABCG2 and ABCB5 act as a potential marker to characterize CSCs in melanoma [3,4]. However, the molecular mechanism between cancer stem cells markers and with their associated functions remains to be elucidated. Studies showed that CD133 has potential to act as an important marker to characterize melanoma initiating cells [5,6]. In our recent study [7], we have identified that CD133<sup>+</sup> subpopulation in melanoma exhibits distinct molecular feature compared with CD133- cells. CD133<sup>+</sup> cells showed higher expression of Oct3/4 and Nanog and exhibit self-renewal properties under in vivo and in vitro conditions which indicates a characteristic feature of CSCs. These cells are highly tumorigenic in nature and maintain long-term tumor growth. Our in vitro and in vivo data showed that CD133<sup>+</sup> subpopulation exhibits EMT, metastasis and angiogenesis in melanoma. Previous studies showed that acquisition of chemoresistant is associated with high expression of multidrug resistant (MDR) protein, IL-8 and VEGF [3,8,9]. Our data indicates that CD133<sup>+</sup> subpopulation exhibits higher percentage of SP phenotypes which probably associated with chemoresistance against DTIC, Dox, Dabrafenib and Trametinib [7]. Osteopontin, a cytokine has multiple role in tumor progression and metastasis [10,11]. The study by Pietras et al. have showed that osteopontin (OPN)-CD44 signaling axis in glioma niche enhances CSCs traits and promotes aggressive tumor growth [12]. OPN also promotes CSCs phenotypes in hepatocellular carcinoma cells via integrin-NF-κB HIF-1α signaling [13]. Additionally, Kumar et al. have observed that stromal OPN induces melanoma progression through

enrichment of SP phenotypes [14]. Angiogenesis is an important phenomenon for the tumor development and metastasis [15]. Our recent data showed that CD133<sup>+</sup> cells exhibit enhance expression of vascular endothelial growth factor (VEGF) and robust angiogenesis either through trans-differentiation into endothelial-like cells or recruiting neo-vascularisation [7]. We also observed that CD133<sup>+</sup> cells exhibit the upregulation of mesenchymal markers Slug, Snail and Ncadherin and downregulation of epithelial markers E-cadherin which is an indication of the EMT phenotype. Additionally, CD133<sup>+</sup> cells are robustly metastasized to the lung as compared to CD133- cells [7].



**Figure 1:** Melanoma is heterogeneous in nature. The CD133+ melanoma cells have both tumor initiating and self-renewal ability. The Notch1-p38-MAPK signaling axis regulates tumor formation properties of the CD133+ cells. Andrographolide (Andro) is able to attenuate this signaling axis in CD133+ cells that leads to abrogation in melanoma progression and metastasis.

Several studies indicate that CSCs are responsible for limited tumor response against conventional treatment due to specific intracellular molecular properties [16]. Thus identification of signaling pathway by which CSCs exhibit protective mechanisms against conventional therapy is essential at this moment. The molecular characterization in our recent studies revealed that Notch1 and MAPK signaling pathway is predominantly active in CD133<sup>+</sup> compared with CD133-cells [7]. Our genetic and pharmacological experimental analysis showed that Notch1 intracellular domain (NICD1) transcriptinally regulates CD133 expression which activates p38-MAPK pathway. Activation of p-38 MAPK pathway leads to AP-1-DNA binding and regulates angiogenesis and metastasis associated genes such as VEGF and MMPs (Figure 1). Several drugs including DTIC, Trametinib and Dabrafenib widely used as chemotherapeutic agents for the treatment of melanoma patients. Moreover, these agents exhibit several side effects and drug resistance [8,17,18]. Studies showed that Andrographolide (Andro), which is derived from Andrographis paniculata, act as an anticancer agent with less side effect and is able to target CSCs in multiple myeloma [19-21]. Our data demonstrate that Andro is able to eliminate CD133<sup>+</sup> cells from melanoma and reduces the tumor growth. Mechanistic study revealed that Andro inhibits Notch1-MAPK signaling axis that leads to attenuation of CD133<sup>+</sup> cells-mediated melanoma progression, metastasis and angiogenesis (Figure 1).

# Conclusion

Our results identify the molecular mechanism of CSCs that help in the metastasis, angiogenesis and tumor progression. Targeting Notch1-MAPK signaling axis attenuates CD133<sup>+</sup> cells-mediated melanoma progression. Thus, our study has addressed an important gap with CSCs markers and their associated function in melanoma model. In future, we need to find potential therapeutic targets and agents for the prevention or treatment of CSCs mediated melanoma progression.

# **Conflicts of Interest**

The authors disclose no potential conflicts of interest.

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# References

- Frank NY, Schatton T, Kim S, Zhan Q, Wilson BJ, et al. (2011) VEGFR-1 expressed by malignant melanoma-initiating cells is required for tumor growth. Cancer Res 71: 1474-1485.
- 2. Li F, Tiede B, Massagué J, Kang Y (2007) Beyond tumorigenesis: cancer stem cells in metastasis. Cell Res 17: 03-14.
- Kumar D, Gorain M, Kundu G, Kundu GC (2017) Therapeutic implications of cellular and molecular biology of cancer stem cells in melanoma. Mol Cancer 16: 7.
- 4. Parmiani G (2016) Melanoma cancer stem cells: markers and functions. Cancers 8: 34.
- Madjd Z, Erfani E, Gheytanchi E, Moradi-Lakeh M, Shariftabrizi A, et al. (2016) Expression of CD133 cancer stem cell marker in melanoma: a systematic review and meta-analysis. Int J Biol Markers 31: e118-125.

- 6. Monzani E, Facchetti F, Galmozzi E, Corsini E, Benetti A, et al. (2007) Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. Eur J Cancer 43: 935-946.
- Kumar D, Kumar S, Gorain M, Tomar D, Patil HS, et al. (2016) Notch1-MAPK signaling axis regulates CD133+ cancer stem cell-mediated melanoma growth and angiogenesis. J Invest Dermatol 136: 2462-2474.
- 8. Lev DC, Ruiz M, Mills L, McGary EC, Price JE, et al. (2003) Dacarbazine causes transcriptional up-regulation of interleukin 8 and vascular endothelial growth factor in melanoma cells: a possible escape mechanism from chemotherapy. Mol Cancer Ther 2: 753-763.
- 9. Vinogradov S, Wei X (2012) Cancer stem cells and drug resistance: the potential of nanomedicine. Nanomed 7: 597-615.
- Ahmed M, Behera R, Chakraborty G, Jain S, Kumar V, et al. (2011) Osteopontin: a potentially important therapeutic target in cancer. Expert Opin Ther Targets 15: 1113-1126.
- Bandopadhyay M, Bulbule A, Butti R, Chakraborty G, Ghorpade P, et al. (2014) Osteopontin as a therapeutic target for cancer. Expert Opin Ther Targets 18: 883-895.
- 12. Pietras A, Katz AM, Ekström EJ, Wee B, Halliday JJ, et al. (2014) Osteopontin-CD44 signaling in the glioma perivascular niche enhances cancer stem cell phenotypes and promotes aggressive tumor growth. Cell Stem Cell 14: 357-369.
- Cao L, Fan X, Jing W, Liang Y, Chen R, et al. (2015) Osteopontin promotes a cancer stem cell-like phenotype in hepatocellular carcinoma cells via an integrin–NF-κB-HIF-1α pathway. Oncotarget 6: 6627-6640.
- 14. Kumar S, Sharma P, Kumar D, Chakraborty G, Gorain M, et al. (2013) Functional characterization of stromal osteopontin in melanoma progression and metastasis. PloS One 8: e69116.
- 15. Mishra R, Kumar D, Tomar D, Chakraborty G, Kumar S, et al. (2015) The potential of class 3 semaphorins as both targets and therapeutics in cancer. Expert Opin Ther Targets 19: 427-442.
- Skvortsov S, Debbage P, Lukas P, Skvortsova I (2015) Crosstalk between DNA repair and cancer stem cell (CSC) associated intracellular pathways. Semin Cancer Biol 31: 36-42.
- Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, et al. (2012) Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med 367: 1694-1703.
- Vorobiof DA, Rapoport BL, Mahomed R, Karime M (2003) Phase II study of pegylated liposomal doxorubicin in patients with metastatic malignant melanoma failing standard chemotherapy treatment. Melanoma Res 13: 201-203.
- 19. Kumar S, Patil HS, Sharma P, Kumar D, Dasari S, et al. (2012) Andrographolide inhibits osteopontin expression and breast tumor growth through down regulation of PI3 kinase/Akt signaling pathway. Curr Mol Med 12: 952-966.
- Zhang Q-Q, Ding Y, Lei Y, Qi C-L, He X-D, et al. (2014) Andrographolide suppress tumor growth by inhibiting TLR4/NF-κB signaling activation in insulinoma. Int J Biol Sci 10: 404-414.
- Gunn EJ, Williams JT, Huynh DT, Iannotti MJ, Han C, et al. (2011) The natural products parthenolide and andrographolide exhibit anti-cancer stem cell activity in multiple myeloma. Leuk Lymphoma 52: 1085-1097.