Targeting the lncRNA SAMMSON reveals metabolic vulnerability in melanoma

Colin R Goding

Ludwig Institute for Cancer Research, Nuffield Department of Medicine, University of Oxford, Headington, Oxford, OX3 7DQ, UK.

Corresponding author: Colin R Goding
Email: colin.goding@ludwig.ox.ac.uk

In a recent study, Leucci et al. report a role for the long non-coding RNA SAMMSON in driving mitochondrial function in melanoma. Targeting SAMMSON, the gene of which is frequently co-amplified with *MITF*, highlights a new cell type-specific therapeutic vulnerability in melanoma irrespective of BRAF, NRAS or p53 status.

Sequencing the human genome revealed far fewer protein coding genes than were previously imagined. Although some of the intergenic space is taken up with regulatory elements that provide the crucial spatio-temporal regulation of mRNA expression necessary for development and homeostasis, the great majority of the genome appeared to lack function. How things have changed. Recent estimations now suggest that at least 75% of the genome is transcribed (Djebali et al., 2012), with non-coding RNAs making up the vast majority of transcripts. These comprise so-called small RNAs (<200 bases), including microRNAs and piRNAs, and long non-coding RNAs (lncRNAs) (>200 bases) that include anti-sense RNAs, overlapping bi-directional transcripts, and those that are intronic or intergenic. The potential regulatory roles of lncRNAs are attracting increasing attention as a major class of biological regulators, with current estimates indicating almost 16,000 lncRNA genes. Broadly speaking lncRNAs fall into two functional classes: those that function in cis, acting at or very close to the site of synthesis, for example by nucleating specific chromatin conformations that facilitate up- or down-regulation of transcription; and those that act in trans and play a more widespread or global role. Importantly, lncRNAs can be inactivated by small interfering RNAs and/or antisense oligonucleotides (ASOs) and therefore represent potential therapeutic targets. However, to date few lncRNAs have a characterized pathophysiological role. The identification by Leucci et al. (2016) of SAMMSON (previously referred to as LINC01212) as a lncRNA with a crucial role in melanoma survival has provided proof-of-principle that targeting a lncRNA in vivo is a potentially viable therapeutic option.

Cutaneous melanoma, a highly aggressive skin cancer, arises from melanocytes that have their developmental origins in the neural crest. Oncogenic transformation of melanocytes to melanoma usually occurs via a combination of senescence bypass, for example by inactivation of the *CDKN2A* locus or loss of *PTEN*, combined with de-regulated MAPK signaling primarily driven by activating mutations in BRAF (50%) or NRAS (20%). Localized early stage melanoma is effectively cured by surgical excision, but metastatic disease poses several challenges. Recently developed immune checkpoint inhibitors can be highly effective in some patients, but a significant proportion of patients either do not respond or develop resistance; treatment with BRAF inhibitors usually results in resistance developing within a few months. Therefore, an urgent need remains for complementary therapeutic strategies.

Like in most cancers, the tumor microenvironment also plays a key role in melanoma by promoting metastatic spread and the development of therapeutic resistance. For melanoma, one of the hallmarks of intra-tumor phenotypic heterogeneity that arises though the influence of the microenvironment is the expression of the microphthalmia-associated transcription factor (MITF), which is required for melanoblast survival during development and melanocyte differentiation. Significantly, MITF also promotes proliferation and suppresses

invasion. Dynamic expression of MITF in response to microenvironmental cues is thought to drive the reversible inter-conversion of cells between differentiated, proliferative and invasive states, a process termed phenotype-switching that underpins disease progression (Hoek and Goding, 2010). The pro-proliferative role of MITF, combined with the observation that the gene can be highly amplified in some melanomas, led to *MITF* being dubbed a lineage-addiction oncogene (Garraway et al., 2005). However, even though *MITF* can be amplified 100-fold, the change in protein levels is restricted to less than 2-fold. One interpretation is that despite amplification of *MITF*, the protein levels must be restricted to prevent cell differentiation. The work from Leucci et al. (2016) provides another possible explanation: the chromosome 3p *MITF* focal amplicon invariably contains the *SAMMSON* lncRNA that lies around 30 kb downstream from *MITF*, and unlike MITF protein, SAMMSON RNA levels did correlate with its gene copy number. Moreover, SAMMSON levels did not vary substantially between MITF-high proliferative cells versus MITF-low invasive cell lines, and was barely detectable in melanocytes or non-invasive vertical growth phase melanomas. However, SAMMSON was readily detected in invasive vertical growth phase melanoma and in migratory melanoblasts but among different tumor types was selectively expressed in melanoma.

SAMMSON transcription appears independent of *MITF*; it has its own promoter marked by the classical promoter-associated H3K4me3 and H3K27ac epigenetic marks and is regulated by SOX10, a lineage restricted transcription factor, but not by MITF. However, since SOX10 also regulates MITF expression and is frequently down-regulated in MITF-low melanomas, it is not clear how *SAMMSON* expression would be maintained in MITF-low invasive melanoma cells or cells in which SOX10 is down-regulated due to BRAF inhibition (Sun et al., 2014). One possibility is that in SOX10-low cells *SAMMSON* is regulated by an alternative SOX factor such as SOX9, known to play a key antagonistic role to SOX10 in melanoma (Shakhova et al., 2015).

Crucially, SAMMSON confers a growth advantage on melanoma cells. Targeting SAMMSON for degradation reduced clonogenicity, irrespective of BRAF, NRAS or p53 status, including in cell lines exhibiting BRAF inhibitor-resistance, but did not affect melanocytes, highlighting the ‘addiction’ of melanomas to *SAMMSON* expression. It also reduced viability/growth of invasive melanoma cells, known to exhibit increased resistance to MAPK therapeutics. Importantly, ectopic expression of SAMMSON in melanoma cells conferred a growth advantage, indicating that SAMMSON acts in trans, an observation consistent with the lack of effect on *MITF* expression following SAMMSON knockdown.

The authors next addressed the role of SAMMSON with an early clue to its mode of action being that a fraction of SAMMSON co-localized with mitochondria. A mass spectrometry approach to identify SAMMSON-interacting proteins not surprisingly revealed

interaction with a number of well-known RNA-binding proteins, including XRN2, a 5'to 3' exonuclease that had previously been detected as a lncRNA-binding factor. However the authors' attention focused on p32, a protein implicated in mitochondrial metabolism and translation of mitochondrially-encoded peptides that is required for proliferation of cancer cells including melanoma (Fogal et al., 2010; Fogal et al., 2008). SAMMSON-p32 interaction was confirmed using RNA-immunoprecipitation. Significantly, depletion of SAMMSON decreased the fraction of p32 associated with mitochondria, increased the nuclear fraction, and markedly diminished mitochondrial membrane potential. Consistent with SAMMSON-p32 interaction being important for mitochondrial function, depletion of SAMMSON phenocopied depletion of p32, yielding structurally aberrant mitochondria (Figure 1). The p53-independent apoptotic response triggered by SAMMSON depletion appeared to arise as a result of accumulation of mitochondrial peptide precursors and mitochondrial import defects, collectively known as mitochondrial precursor-over-accumulation stress (mPOS).

Using patient-derived xenograft models, the authors were able to demonstrate that intravenous administration of a SAMMSON-specific antisense oligonucleotide decreased tumor growth and synergized with Dabrafenib, a BRAF inhibitor, to induce apoptosis in vivo. The synergy observed is likely to arise because BRAF inhibition activates an MITF-PGC1α axis to elevate mitochondrial oxidative phosphorylation (Haq et al., 2013), rendering cells more susceptible to inhibition of mitochondrial function by targeting the SAMMSON-p32 complex. Whether targeting SAMMSON will be effective in metastatic disease and whether it will be more effective than using other mitochondrial targeting agents, which unlike SAMMSON would not be melanoma specific, remains to be determined. Nevertheless, the work from Leucci et al. (2016) reveals a key role for the SAMMSON lncRNA that may explain how the *MITF* amplicon impacts melanoma proliferation. It also nicely demonstrates how targeting a key lncRNA can have therapeutic benefit, at least in non-metastatic xenograft models, and reinforces other studies (Zhang et al., 2016) showing how targeting mitochondrial function, and especially mitochondrial protein folding, is likely to prove beneficial in melanoma.

Djebali, S., Davis, C. A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., Tanzer, A., Lagarde, J., Lin, W., Schlesinger, F., et al. (2012). Landscape of transcription in human cells. Nature *489*, 101-108.

Fogal, V., Richardson, A. D., Karmali, P. P., Scheffler, I. E., Smith, J. W., and Ruoslahti, E. (2010). Mitochondrial p32 protein is a critical regulator of tumor metabolism via maintenance of oxidative phosphorylation. Mol. Cell. Biol. *30*, 1303-1318.

Fogal, V., Zhang, L., Krajewski, S., and Ruoslahti, E. (2008). Mitochondrial/cell-surface protein p32/gC1qR as a molecular target in tumor cells and tumor stroma. Cancer Research *68*, 7210-7218.

Garraway, L. A., Widlund, H. R., Rubin, M. A., Getz, G., Berger, A. J., Ramaswamy, S., Beroukhim, R., Milner, D. A., Granter, S. R., Du, J., et al. (2005). Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature *436*, 117-122.

Haq, R., Shoag, J., Andreu-Perez, P., Yokoyama, S., Edelman, H., Rowe, G. C., Frederick, D. T., Hurley, A. D., Nellore, A., Kung, A. L., et al. (2013). Oncogenic BRAF regulates oxidative metabolism via PGC1alpha and MITF. CancerCell *23*, 302-315.

Hoek, K., and Goding, C. R. (2010). Cancer stem cells versus phenotype switching in melanoma. Pigment Cell Melanoma Res *23*, 746-759.

Leucci, E., Vendramin, R., Spinazzi, M., Laurette, P., Fiers, M., Wouters, J., Radaelli, E., Eyckerman, S., Leonelli, C., Vanderheyden, K., et al. (2016). Melanoma addiction to the long non-coding RNA SAMMSON. Nature *531*, 518-522.

Shakhova, O., Cheng, P., Mishra, P. J., Zingg, D., Schaefer, S. M., Debbache, J., Hausel, J., Matter, C., Guo, T., Davis, S., et al. (2015). Antagonistic cross-regulation between Sox9 and Sox10 controls an anti-tumorigenic program in melanoma. PLoS Genet *11*, e1004877.

Sun, C., Wang, L., Huang, S., Heynen, G. J., Prahallad, A., Robert, C., Haanen, J., Blank, C., Wesseling, J., Willems, S. M., et al. (2014). Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. Nature *508*, 118-122.

Zhang, G., Frederick, D. T., Wu, L., Wei, Z., Krepler, C., Srinivasan, S., Chae, Y. C., Xu, X., Choi, H., Dimwamwa, E., et al. (2016). Targeting mitochondrial biogenesis to overcome drug resistance to MAPK inhibitors. J Clin Invest. http://dx.doi.org/10.1172/JCI82661

Figure 1. Inhibition of SAMMSON reveals metabolic vulnerability in melanoma. The *SAMMSON* lncRNA gene is co-amplified with *MITF* in melanoma and the SAMMSON-p32 complex is required for correct mitochondrial biogenesis. Depletion of SAMMSON leads to stress associated with accumulation of mitochondrial peptide precursors and mitochondrial import defects and consequently p53-independent apoptosis. BRAF inhibitors (BRAFi) promote dependency on mitochondrial oxidative phosphorylation and consequently synergize with SAMMSON inhibition.