Editorial

Emerging role of RNA binding protein UNR/CSDE1 in melanoma

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The prognosis of metastatic melanoma remains poor despite recent advances in targeted and immunotherapy. Deciphering the mechanisms of tumor invasion and metastasis, and elucidating new targets for drug development could substantially improve survival in patients with metastatic melanoma. In this regard, RNA binding proteins (RBP) play an important role in RNA metabolism and are drawing considerable attention as drivers of oncogenesis and therapeutic targets. RNA binding proteins synchronize with the target RNA to play a key role in the regulation of cellular processing and are important for gene transcription and post-translational regulation (1). Wurth *et al.* recently published an article in Cancer Cell exploring the role of Upstream-of-N-Ras (UNR) in invasion and progression of melanoma cells (2). The *UNR* gene was identified as a transcription unit located immediately upstream of N-Ras in the genome of several mammalian species (3). UNR protein consists of five cold-shock domains (CSDs). These domains bind single stranded DNA and RNA and consist of ~70 amino-acid residues (4). CSD containing proteins are involved in transcriptional and post-transcriptional control of gene expression. Experiments in *Drosophila* species have shown the role of UNR in regulating translation of oncogenic transcripts (5).

Wurth *et al.* performed a series of *in vitro* and *in vivo* experiments to demonstrate that UNR plays an oncogenic role in melanoma progression. The authors performed western blot of UHR and immunohistochemistry (IHC) in human melanoma cell lines and benign melanocytes, and showed that levels of UNR are elevated in primary as well as metastatic lesions compared with non-tumoral melanocytes. The authors examined loss-of-function by using an inducible short hairpin RNA (shRNA) system to deplete UNR from human melanoma cell line. Interestingly, they observed reduction in cell numbers in UNR-depleted cells that remained attached to the surface when cells reached confluence while control cells were found to grow as spheres. When cultured in suspension, UNR-depleted cells failed to grow and became apoptotic. In addition, gain-of-function studies with enforced expression of UNR in malignant cells demonstrated increased colony formation even in cells in suspension, suggesting promotion of anchorage-independent growth. Cells that become anchorage independent have the potential to metastasize and colonize distant tissues (6). To explore the generalizability of UNR, the authors evaluated other tumor cells including breast and ovarian cancer, and found that depletion of UNR decrease anoikis resistance and invasive capacities of cells. These pro-oncogenic features of UNR were further verified *in vivo* using xenograft models in mice. Subcutaneous or intravenous injection of UNR-depleted human melanoma cells resulted in smaller tumors or decreased metastasis, respectively, compared with controls. Collectively, the authors showed that UNR is associated with the invasiveness and metastatic property of melanoma cells *in vitro* and *in vivo*.

Next, Wurth *et al.* adopted a systematic approach to identify the molecular mediators of UNR-elicited melanomagenesis using (I) iCLIP (individual nucleotide-resolution crosslinking immunoprecipitation) analysis to identify all of UNR target RNAs and sites of binding; (II) RNA-seq analysis to detect changes in mRNA steady-state

levels after silencing UNR; and (III) ribosome profiling to identify changes in relative association of ribosomes and hence translational regulation.

Two independent iCLIP experiments identified 1,532 UNR common targets, most of which are protein-coding RNAs. Of these, 18.5% were found to be overlapped with *Drosophila* UNR targets indicating conserved functions of UNR. Independent RNA immunoprecipitation analysis revealed for the first time that 26% of the UNR targets code for cancer-related factors. UNR also plays a prevalent role as an activator of mRNA accumulation. UNR binds to the 5' untranslated region (UTR), often at internal ribosome entry site (IRES), sometimes at the coding region and often at 3'UTR resulting in transcriptional and translational effects. Authors found that UNR depletion consistently down-regulated tumor-promoting factors while tumor-suppressing factors were up-regulated.

They performed ribosome profiling and identified that 451 genes including 127 UNR iCLIP targets are regulated at the translation level. Of the 127 direct UNR targets, 60 (47%) showed changes only in ribosome distribution without changes in RPF levels, suggesting that UNR regulates translation of its melanoma targets at the level of translation elongation/termination. Next, the authors chose to explore VIM (Vimentin) and RAC1 (Ras-related C3 botulinum toxin substrate 1) that are highly altered in melanoma and are important for invasion and metastasis (7,8) as potential targets of UNR. Western Blot analyses revealed strong down-regulation of VIM and RAC1 in the absence of UNR and in the presence of proteasome inhibitors, indicating that UNR plays a key role in the synthesis of these proteins. Ribosome profiling was also performed to provide information about ribosomes along the transcripts. Hunt *et al.* showed that UNR is a factor required for internal initiation of translation which allows cap-independent recruitment of ribosomes to mRNA, mediated by sequences called internal ribosome entry sites (IRESs) (9). UNR plays a role in the elongation of VIM and RAC1 proteins. Reporter assays and transfection assays confirmed that UNR upregulates VIM at the level of translation by binding to the 3' UTR. *VIM* mRNA encodes VIM, a component of the intermediate filaments that protect melanoma cells as they change morphology, and key marker of epithelial-mesenchymal transition (EMT) (10). *RAC1* mRNA encodes a GTPase in the RAS superfamily which functions in cell signaling, proliferation, and cytoskeletal architecture. RAC1 promotes melanoma metastasis by promoting invadopodia formation (11). Taken together, these analyses showed that UNR regulates critical melanoma genes at the level of translation.

The authors also performed a network analysis to identify candidate downstream effectors of UNR in melanoma progression. Melanoma-relevant genes were retrieved and intersected with UNR-regulated iCLIP targets to build a network of interaction data that included physical interactions, co-regulation, and molecular modifications. They found that 66% of genes highly mutated in melanoma and/or previously shown to control tumorigenic features of melanoma cells are connected directly or indirectly with UNR targets. This analysis not only identified 15 genes out of 45 UNR targets in the network but also other targets, currently unknown to play a role in melanoma. Experiments again showed that UNR depletion reduced the levels of VIM and RAC1 while also increasing the levels of PTEN. Depletion of UNR reduced the number and size of colonies that grow in soft agar, while, on the other hand, VIM and RAC1 over-expression fully restored the colony growth and number.

UNR as a RBP is a fundamental player in RNA metabolism, and its contribution to diseases has been recently recognized. The article by Wurth and colleagues explores the role played by UNR in melanoma invasion and metastasis. Both *in vitro* and *in vivo* experiments have shown that UNR promotes anoikis resistance, migration and invasion in cultured melanoma cells. Translation elongation is an important pro-oncogenic mechanism and UNR, which activates rather than represses elongation of VIM and RAC1 mRNAs, underscores its oncogenic potential for this reason as well. UNR downregulates *PTEN* (a tumor-suppressor) and upregulates *CCL2* (an oncogene). RNA regulons are additionally coordinated by UNR to promote invasion and metastasis.

Wurth and colleagues should be congratulated for their elegant work that has identified the central post-transcriptional role RBP UNR plays in melanoma survival, invasion and metastasis, as well as the down stream targets of this pathway, Wurth *et al.* have identified novel therapeutic targets and potential biomarkers of outcome that will enhance future strategies.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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