# A wound-healing program is hijacked to promote cancer metastasis

**In this issue of JEM, Sundaram et al. (https://doi.org/10.1084/jem.20170354) report a mechanism by which the normal epithelial wound healing response is "hijacked" to promote invasion and metastasis in head and neck squamous carcinomas (HNSCCs), a finding that unveils new markers of poor outcomes and potential targets for therapeutic intervention.**

Repair of epithelial wounding involves the transient activation of programs for cellular migration and proliferation as well as tissue matrix remodeling (Gurtner et al., 2008). These wound-healing programs overlap highly with pathways activated in cancer cells and are therefore under exquisite control, thus ensuring that the wound response is self-limited. This homeostasis is maintained in part by microRNA (miR) networks, which serve as molecular switches to regulate initiation and limitation of wound healing programs (Horsburgh et al., 2017). Previously, Sundaram et al. (2013) described a unique example of such a mechanism involving miR-198, which functions to attenuate the wound-healing response and, remarkably, is embedded within the transcription unit for the pro-migratory factor follistatin-like 1 (*FSTL1*). Expression of miR-198 is high in normal epidermis but is down-regulated upon wounding, accompanied by a reciprocal rise in *FSTL1*. The mechanistic lynchpin for this "see-saw" regulation of miR-198 and *FSTL1* is transforming growth factor-β (TGF-β) signaling, which is activated in response to wounding and mediates suppression of the KH-type splicing regulatory protein KSRP, an RNA-binding factor whose presence is required for miR-198 processing. Thus, wounding activates TGF-β to inhibit miR-198, increase *FSTL1*, and thereby promote keratinocyte migration and tissue remodeling (see figure; Sundaram et al., 2013).

The initial report of the authors linked a failure to activate this response to nonhealing diabetic ulcers. In contrast, the new work describes aberrant activation of FSTL1 and down-regulation of miR-198, in this case via epidermal growth factor (EGF)–dependent suppression of KSRP, as a driver of metastasis in head and neck squamous cell carcinomas (HNSCCs; see Sundaram et al. in this issue). This paper further elaborates on the downstream mechanisms involved, showing that FSTL1 promotes expression of the matrix metalloprotein MMP9, which can degrade extracellular matrix, through an effect on Wnt and mitogen-activated protein kinase (MAP kinase) signaling. Additionally, Sundaram et al. (2017) find that the miR-198 target DIAPH, which is known to promote migration, is increased in this setting. The physiological relevance of these findings is supported by in vivo data the authors provide showing that knockdown of FSTL1 and DIAPH collaboratively block pulmonary metastases in a tail vein injection model and by clinical data showing that HNSCC patients whose tumors express high levels of both factors have a very poor prognosis (Sundaram et al., 2017).

These and recent related findings are beginning to yield a deeper understanding of the biology of HNSCC, a highly lethal malignancy of the upper aerodigestive tract for which relatively few advancements have been made in improving outcomes in recent decades. Although typically diagnosed as a local condition, most patients ultimately succumb as a result of metastasis, and therefore a detailed understanding of the mechanisms involved may yield important therapeutic opportunities. EGFR has long been considered an important oncogenic driver in HNSCC, and the work by Sundaram et al. (2017) demonstrates yet another mechanism by which activation of this receptor contributes to pathogenesis in this disease. Unfortunately, therapeutic targeting of the EGFR pathway

**Insight from Leif W. Ellisen**

has been a relative disappointment to date, despite numerous clinical trials involving both antibodies and small molecules that inhibit EGFR itself (Sharafinski et al., 2010). Accordingly, only one agent, the monoclonal antibody cetuximab, is currently FDA approved for treatment of locally advanced or recurrent/metastatic HNSCC. New, more potent drugs and combinations that target EGFR itself may eventually overcome the limitations of this approach (Hammerman et al., 2015). In this context, it will be of interest to determine whether the factors identified by Sundaram et al. (2017), including FSTL1 and DIAPH, are inhibited after clinical EGFR inhibition and, if so, whether these effects correlate with therapeutic benefit. Nonetheless, the limited success achieved with direct receptor inhibition to date makes clear the need for innovative approaches that target additional players in the EGFR pathway and other cooperating mechanisms.

The work by Sundaram et al. (2017) builds on a host of other studies that implicate the wound-healing response and miR-dependent pathways in poor outcomes in HNSCC. For example, properties of migration and tissue remodeling that characterize wound healing are also

Leif W. Ellisen, Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, MA: lellisen@mgh.harvard.edu

prominent in cells that undergo epithelial-to-mesenchymal transition (EMT). In HNSCC, an EMT phenotype is associated with stem-like characteristics, poor outcomes, and an uncommon subtype of the disease known as spindle cell carcinoma (Graves et al., 2014). Although a full explanation of the mechanisms leading to EMT in HNSCC is lacking, multiple pathways including TGF-β and FGFR are likely to collaborate and interact with EGFR signaling to mediate this transformation (see figure; Nguyen et al., 2013). Accordingly, nascent therapeutic efforts in this area have shown some promise. Of note, neither *EGFR* overexpression nor genomic amplification is correlated with response to EGFR inhibitors in HNSCC. In contrast, clinical responses to FGFR inhibitors have been observed in HNSCC patients whose tumors show amplification, mutation, or fusion of various *FGFR* genes (Hammerman et al., 2015). Furthermore, modulating TGF-β signaling with retinoids has long been tested as potential preventatives for oral cancers, and preclinical data suggest that retinoids can also have potent effects on established tumor cells (Graves et al., 2014).

The existence of a network connecting wound healing, EMT, TGF-β, and the key miR-dependent program described by Sundaram et al. (2017) is further supported by recent work on key transcription factors implicated in HNSCC. SOX2 is a pluripotency factor subject to frequent genomic amplification in HNSCC, where its high-level expression promotes an epithelial phenotype and cell survival (Thierauf et al., 2017). However, loss of SOX2 in late-stage tumors is a poor prognostic, a finding attributed to the induction of EMT and activation of a wound-healing program (Bayo et al., 2015). SOX2 is located on chromosome 3q, and its amplification in HNSCC is associated with that of p63, another transcription factor and master epithelial regulator that is not only coexpressed but also physically associated with SOX2 in squamous carcinoma cells (Watanabe et al., 2014). Our own work showed that p63 controls TGF-β signaling in tumors via additional upstream miRs, thereby suppressing KSRP and miR-198 and promoting metastatic tumor dissemination, all in keeping with the wound-healing response delineated by Sundaram et al. (2017) (Rodriguez Calleja et al., 2016). In addition, reciprocal regulatory interactions have been demonstrated between p63 and EGFR signaling in HNSCC and breast cancer, which provides another point of connection with the new findings (Holcakova et al., 2017). Although the involvement of SOX2 and p63 in this context may not be immedi- ately therapeutically actionable, emerging data on control of the epigenome by these central transcription factors alludes to the potential viability of future epigenetic therapy approaches for refractory HNSCC (Alexandrova et al., 2013).

Perhaps the most prominent message of the study by Sundaram et al. (2017) is to underscore the fundamental contribution of miRs to all the major metastasis-associated cellular properties, including migration, invasion, and cytoskeletal remodeling. Numerous studies of miR-dependent pathways in HNSCC support this view (Denaro et al., 2014). These include the finding that miR expression can predict the presence of lymph node metastasis, a key prognostic in HNSCC (de Carvalho et al., 2015), as well as disease recurrence (Citron et al., 2017). In particular, miR-198 has emerged as a central control point for multiple nodes that contribute to tumor progression and metastatic dissemination in several cancer contexts. As such, strategies aimed at restoration of this factor could in the future represent a means of selective metastasis-suppressive therapy. Clinical trials of direct miR targeting as a therapeutic strategy are now ongoing, and they may have the most near-term application in cancers such as HNSCC, which are anatomically highly accessible, allowing direct introduction of therapeutic agents. Alternatively, a focus on strategies to manipulate the genetic and epigenetic regulatory mechanisms of the miRs themselves, rather than on their myriad downstream targets, may also prove successful in this context (see figure).

Why might miR-198 play such a prominent role in both wound healing and cancer pathogenesis? An intriguing speculation comes from the fact that this miR is primate specific, a finding that implies a role beyond that in an evolutionarily conserved wound-healing program. Like many well-documented examples of cancer-associated miRs, miR-198 is implicated in tumor suppressive functions. Also reminiscent of other cancer-associated miRs, miR-198 modulates the same pathway as the coding transcription unit (*FSTL1*) in which it is embedded (Sundaram et al., 2013). Collectively, these observations suggest
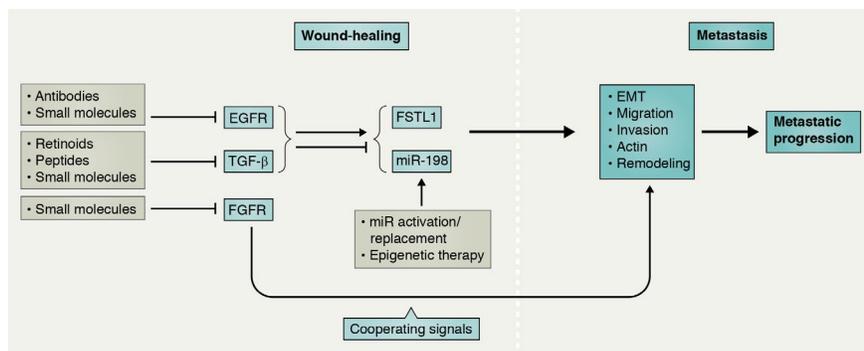


**Mechanisms of wound healing and therapeutic interventions linked to metastatic tumor progression. Key signaling pathways implicated in the wound-healing response and epithelial-to-mesenchymal transition (EMT) are shown at left. In response to wounding, upstream signals inhibit miR-198 processing and coordinately promote FSTL1 expression. Deregulation of these upstream signals disrupts this homeostatic mechanism and, together with related pathways (e.g., FGFR), promotes metastatic progression in HNSCC and other cancers. Shown in grey boxes are ongoing pharmacologic approaches to inhibit these pathways in human cancer.**

that the recent appearance of such miRs could reflect an evolutionary mandate for tight control of an ancient but essential process (i.e., wound healing) that is governed by the gene in which they are embedded (Koufaris, 2016). Deregulation of such homeostatic mechanisms, as described in the work of Sundaram et al. (2017), promotes carcinogenesis and tumor progression. These findings therefore highlight a central role for this recently evolved, miR–dependent homeostatic mechanism, and they underscore the degree to which regenerative tissue healing and cancer can be viewed as two sides of the same coin.

## REFERENCES

Alexandrova, E.M., et al. 2013. *Cell Death Differ.* http://dx.doi.org/10.1038/cdd.2013.122

Bayo, P., et al. 2015. *Mol. Oncol.* http://dx.doi.org/10.1016/j.molonc.2015.05.006

Citron, F., et al. 2017. *Clin. Cancer Res.* http://dx.doi.org/10.1158/1078-0432.CCR-16-2814

de Carvalho, A.C., et al. 2015. *BMC Med.* http://dx.doi.org/10.1186/s12916-015-0350-3

Denaro, N., et al. 2014. *Anticancer Res.* 34:6887–6896.

Graves, C.A., et al. 2014. *Clin. Transl. Med.* http://dx.doi.org/10.1186/s40169-014-0039-9

Gurtner, G.C., et al. 2008. *Nature.* http://dx.doi.org/10.1038/nature07039

Hammerman, P.S., et al. 2015. *Cancer Discov.* http://dx.doi.org/10.1158/2159-8290.CD-14-1205

Holcakova, J., et al. 2017. *Breast Cancer Res. Treat.* http://dx.doi.org/10.1007/s10549-017-4216-6

Horsburgh, S., et al. 2017. *Clin. Sci.* http://dx.doi.org/10.1042/CS20170039

Koufaris, C. 2016. *BioEssays.* http://dx.doi.org/10.1002/bies.201500135

Nguyen, P.T., et al. 2013. *Br. J. Cancer.* http://dx.doi.org/10.1038/bjc.2013.550

Rodriguez Calleja, L. et al. 2016. *Cancer Res.* http://dx.doi.org/10.1158/0008-5472.CAN-15-2317

Sharafinski, M.E., et al. 2010. *Head Neck.* http://dx.doi.org/10.1002/hed.21365

Sundaram, G.M., et al. 2013. *Nature.* http://dx.doi.org/10.1038/nature11890

Sundaram, G.M., et al. 2017. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20170354

Thierauf, J., et al. 2017. *Cancers (Basel).* http://dx.doi.org/10.3390/cancers9070076

Watanabe, H., et al. 2014. *J. Clin. Invest.* http://dx.doi.org/10.1172/JCI71545