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Grant Title: The Novel Cell Surface Hyaluronidase TMEM2 in Tumor Cell Invasion


**Abstract**

*Objectives:* Tumor invasion involves the breaching of tissue barriers by cancer cells. Degradation of extracellular matrix (ECM) is critical for this process, and past studies revealed critical roles for matrix metalloproteases in tumor invasion. However, research in this field have largely ignored the fact that the tumor ECM also contains high levels of hyaluronan (HA). The unique biophysical and biochemical properties of HA endow this huge polysaccharide with a large hydrodynamic volume and also enable it to form a matrix that acts as a barrier to invading tumor cells. HA accumulation in tumor stroma also elevates intra-tumoral fluid pressure, which further restricts cell movement. Thus, it is thought that tumor cells have the ability to degrade HA to facilitate their invasion through HA-rich stromal tissues. The identity of the hyaluronidase responsible for this process, however, has been elusive. We have recently demonstrated that TMEM2, a transmembrane protein of previously unknown function, is such a cell surface hyaluronidase. In this project, we investigate the functional significance of TMEM2 in tumor cell adhesion and migration.

*Methods:* We used *in situ* HA degradation assays to characterize the ability of a variety of tumor cells to degrade substrate-bound HA and a battery of *in vitro* assays to determine the function of TMEM2 in tumor cell adhesion and migration on HA-rich substrate. Expression of TMEM2 in tumor cells was manipulated by transfection of siRNA and truncated TMEM2 constructs.

*Results:* We found that a variety of tumor cells exhibit the ability to eliminate substrate-bound HA in a tightly localized pattern corresponding to the distribution of focal adhesions (FAs) and stress fibers. Immunostaining of these cells for vinculin reveals that the spots of HA degradation coincide with FAs. Knockdown of TMEM2 almost entirely inhibited *in situ* HA degradation. On the other hand, knockdown of other hyaluronidases (HYAL1, HYAL2, and KIAA1199) had little effect on *in situ* HA degradation, indicating that TMEM2 is the hyaluronidase primarily responsible for contact-dependent HA degradation. mCherry-tagged TMEM2 exhibits overlapping colocalization with both vinculin-positive puncta and sites of HA removal. TMEM2 depletion attenuates the ability of U2OS cells to attach, migrate, and form FAs on HA-containing substrates. Importantly, TMEM2 directly binds at least two integrins, namely α5β1 and αLβ2 (LFA-1). This interaction is demonstrated by cell surface cross-linking/coimmunoprecipitation assays and by direct binding assays with recombinant integrins and TMEM2. The integrin-TMEM2 interaction is mediated by interaction between extracellular domains of respective proteins. Our findings demonstrate a critical role for TMEM2-mediated HA degradation in the adhesion and migration of cells on HA-rich ECM substrates and provide novel insight into the early phase of FA formation.


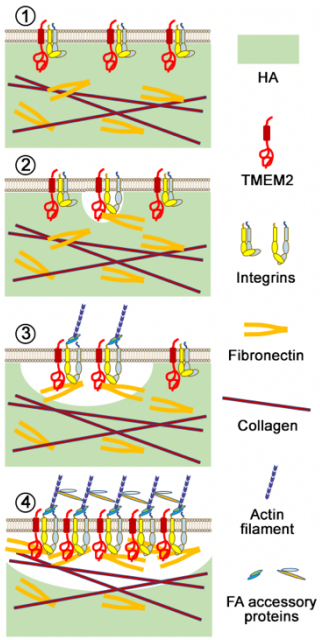


**Figure 1. A model for the role of TMEM2 in integrin-mediated cell adhesion and FA formation.** *1*, High levels of HA in the ECM are inhibitory to the direct engagement of integrins to their ECM ligands. *2*, In the presence of TMEM2, HA in the ECM is locally removed, which generates a microenvironment that is permissible to the direct integrin–ECM engagement. *3*, The association between TMEM2 and integrins promotes the FA formation and maturation via further removal of HA in the vicinity of the integrin–ECM engagement. *4*, This in turn facilitates integrin clustering, integrin-mediated downstream signaling, and cellular responses.