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**Evaluation of the sequence-specific peptide-binding activity of AGR2 has**

**identified specific interaction sites on the oncogenic receptor EpCAM**

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**ABSTRACT**

Anterior gradient-2 (AGR2) is an oncogenic endoplasmic reticulum (ER)-resident protein disulfide isomerase. AGR2 is overexpressed in diverse human cancers and exhibited a pro-metastatic protein essential to cancer progression, drug resistance and metastatic development. The exact mechanism of how AGR2 involves in cancer and how it is regulated is poorly understood. In this study, we aim to expand AGR2 interactome to further unravel its functions. AGR2 protein has a relatively unique property for a chaperone in which it can bind to a sequence-specific peptide motif (TTIYY). Here, we report that the synthetic TTIYY-containing peptide column can affinity-purify AGR2 from crude lysates highlighting peptide selectivity in complex mixtures and this can selectively drive some of its protein-protein interaction functions. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) localized the dominant region in AGR2 that interacts with the TTIYY peptide to within a structural loop from amino acids 131-135 (VDPSL). A peptide binding site consensus of Tx[IL][YF][YF] was developed for AGR2 by measuring its activity against an alanine mutagenized synthetic peptide library. Screening the human proteome for proteins harboring this consensus motif revealed an enrichment in transmembrane proteins and we focused on validating EpCAM as one such oncogenic protein. Recombinant AGR2 and EpCAM proteins formed a dose-dependent protein-protein interaction in-vitro. Proximity ligation assays demonstrated that endogenous AGR2 and EpCAM protein associate in cells. Introducing a single alanine mutation in EpCAM at position Tyr251 attenuated its binding to AGR2 in-vitro and in cells. HDX-MS was used to identify a stable binding site for AGR2 on EpCAM, adjacent to its TLIYY motif and surrounding EpCAM’s detergent binding site. Additionally, immunohistochemistry on tissue microroarrays (TMA) containing 91 cores of oesophageal adenocarcinoma tissues showed that AGR2 and EPCAM were highly expressed. Together, our data define a dominant peptide-binding site on AGR2 that mediates its specific peptide-binding function. Our findings may also provide physiological and clinical context to further dissect the AGR2-EpCAM pathway control in relation to carcinogenesis in oesophageal adenocarcinomas.