A peptide targeting FGFR2 receptor promises early detection of esophageal adenocarcinoma

Review Article

The incidence of esophageal adenocarcinoma (EAC) is rapidly increasing, with 450,000 new cases diagnosed and 400,000 deaths reported annually worldwide. Early detection is complicated by difficulty in detecting the flat premalignant lesions. However, the overexpression of cell surface fibroblast growth factor receptor 2 (FGFR2) is an early event in disease progression. A team comprising researchers based at the University of Michigan, USA and The Fourth Military Medical University, China, have identified a peptide that binds with high specificity to the extracellular domain of FGFR2, making this peptide a promising clinical imaging agent for early detection of esophageal adenocarcinoma.

FGFR2 as a target for early detection of esophageal cancer

The rising incidence of esophageal cancer has been attributed to an increase in obesity and chronic gastroesophageal reflux disease that stimulates a progression from Barrett's esophagus through several stages to EAC (see Figure 1). Low-grade dysplasia (LGD) represents increased risk for EAC and overexpression of FGFR2 starts at this stage.

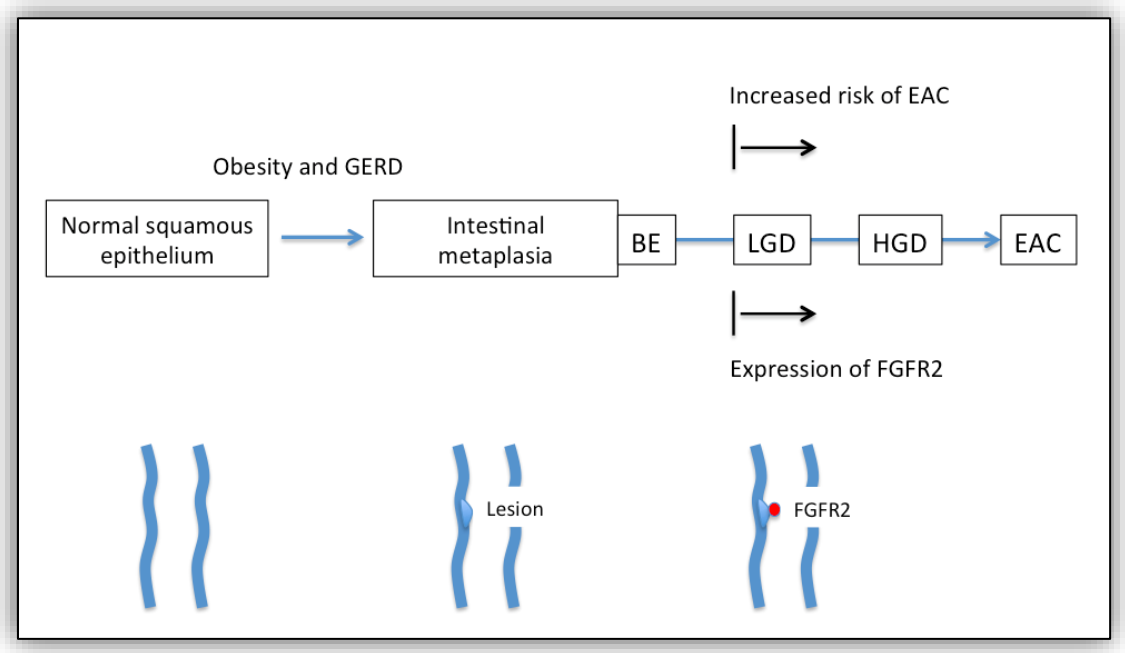

Figure 1. The development of esophageal adenocarcinoma (EAC). Obesity and chronic gastroesophageal reflux disease (GERD) stimulate the replacement of normal squamous epithelium in the esophagus by the intestinal

metaplasia of Barrett's esophagus (BE), followed by successive progression through Low-Grade Displasia (LGD) and High-Grade Displasia (HGD), to EAC.

The FGFR2 receptor is a member of the receptor tyrosine kinases (RTKs), which are involved in cell signaling during cancer progression and are located in the cell membrane. This localization makes FGFR2 particularly accessible for *in vivo* imaging.

The power of peptides in diagnostics and therapy

Peptides have clear advantages in diagnostics, yielding high specificity and binding affinity ligands that can be readily labeled with fluorophores for imaging purposes. In the case of Barrett's neoplasia, which involves only a few centimeters of the esophagus, these properties mean that topical administration of a peptide could be effective, with rapid binding and minimal risk for toxicity. These advantages stimulated the research team to search for a peptide that would be effective in specifically targeting FGFR2, which would make it a powerful tool in early cancer detection.

Phage display yields peptide specific for FGFR2

The first step involved selecting high-affinity peptides directed against the exposed receptor by using purified FGFR2-ECD (Figure 2) to biopan for target-specific peptide sequences using phage display. The research team identified one promising sequence, SRRPASFRTARE, that appeared 15 times in 50 clones analyzed.

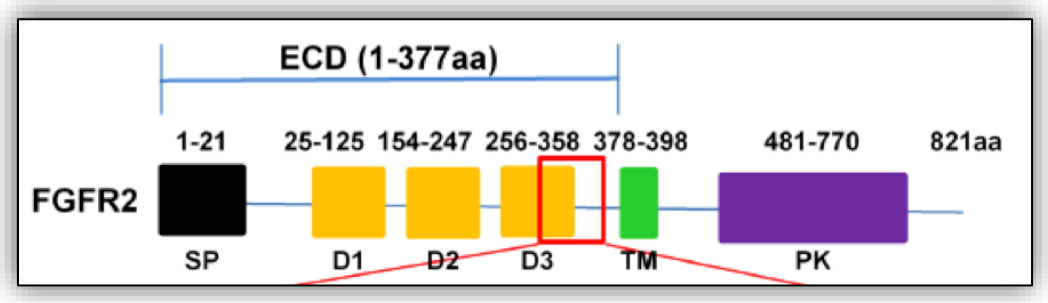

Figure 2. FGFR2 extracellular domain (ECD) contains a signal peptide (SP) and three extracellular immunoglobulin-like domains (D1–D3). A hydrophobic transmembrane region (TM) anchors FGFR2-ECD to the cytoplasmic domain, which contains a tyrosine kinase catalytic domain (PK). The red box indicates a region of alternative splicing. From Supplementary Figure 2A, Zhou et al, 2017.

Peptide synthesis

Cy5.5-labeled forms of the peptide SRRPASFRTARE (called SRR) and the control scrambled form, SPS, were synthesized using standard Fmoc-mediated solid-phase synthesis on rink amide MBHA resin using a PS3® peptide synthesizer. The C-terminal lysine was incorporated as Fmoc- Lys (ivDde)-OH, and the N-terminal amino acid was incorporated with Boc protection to avoid unwanted Fmoc removal during deprotection of the ivDde moiety prior to fluorophore labeling. After assembly, the ivDde side chain-protecting group was removed and the peptide was manually labeled using Cy5.5-NHS ester and purified by prep-HPLC with a C18 column. Cy5.5 was chosen for its high photostability and quantum yield in near-infrared spectrum.

The final purity of the peptides was confirmed with an analytical C18-column, and further characterized using ESI or Q-TOF mass spectrometry.

Peptide is specific for FGFR2

The specificity of SRR*–Cy5.5 could be confirmed using human immortalized BE (QhTERT) cells that express FGFR2. Using unlabeled SRR* as a competitor, the team could also show that the peptide itself bound, rather than the fluorophore. The apparent dissociation constant, k_d, was 68 nM, and the apparent association time constant of 0.16 min^{-1} indicated a time scale of approximately six minutes for onset of binding.

Peptide can be used to detect esophageal cancer and gastric cancer

Confocal microscopy was used to confirm that SRR*–Cy5.5 bound strongly to HGD and EAC cells and minimally to squamous cells and BE cells in sections of human esophagus *ex vivo*. Analysis of fluorescence intensity indicated that the peptide could detect Barrett's neoplasia (HGD and EAC) at a sensitivity of 87% and specificity of 70%, with a target-to-background ratio of 3.0 when compared with pathology. The peptide also bound strongly to sections of human esophageal squamous cell cancers (SCC) and human gastric cancer *ex vivo*.

A promising agent for diagnostics and even therapy

The specificity of SRR*–Cy5.5 clearly indicates the promise of this peptide as a clinical imaging agent for the early detection of EAC and other epithelial-derived cancers. Additionally, such a peptide could be a useful payload carrier for target-directed drug delivery in cancer treatment.

Reference

Identification and validation of FGFR2 peptide for detection of early Barrett's neoplasia. Zhou J, et al. Oncotarget. 2017 Aug 1;8(50):87095-87106. doi: 10.18632/oncotarget.19764. eCollection 2017 Oct 20.

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