Molecular Imaging of Gastric Neoplasm by Targeting EGFR with Confocal Laser Endomicroscopy

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1. Case presentation

A 59-year old woman underwent gastroscopy due to dyspepsia. A 3 cm-sized slightly elevated lesion with erythematous mucosa change was observed at the pyloric channel.

2. Diagnosis

Adenocarcinoma, poorly differentiated

3. Therapy and Clinical course

The laparoscopic radical subtotal gastrectomy with gastrojejunostomy was performed. A specimen of gastric cancer operation is incubated with labeled anti-EGRF antibody for 10 minutes at room temperature. Then, the confocal laser endomicroscopy and immunohistochemical method was used to detect the expression of EGFR. And we can find the correlation between different grades of imunohistochemical result and semi-quantitative assessment of in EGFR expression using confocal laser endomicroscopy.

4. Conclusion

On-site evaluation of cell-surface receptor expression level using confocal laser endomicroscopy enable provide a theoretical basis for the targeted therapy for gastric cancer.

Key Words: Gastric cancer, EGFR, Confocal laser endomicroscopy, Targeted therapy

REFERENCES

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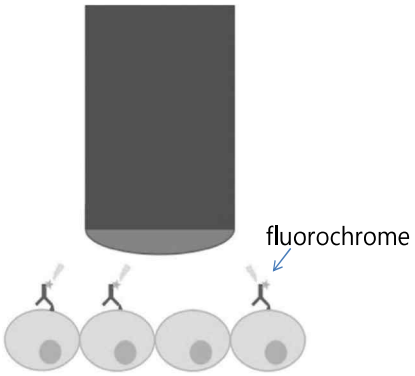
Molecular Imaging

특수한 분자 신호에 근거하여 질병 특이적인 형태적/기능적 조직 변화를 최소 침습적으로 시각화 하는 방법들

Modalities that enable minimally-invasive visualization of disease-specific morphologic or functional tissue alterations based on the specific molecular signature of single cells or whole tissue.



M Goetz and TD Wang. Gastroenterology. 2010 March;138(3):828-33

Applications

- Wide field techniques for the detection of lesions
- Microscopic techniques for in vivo characterization
  - Molecular characterization of neoplasm
  - Assessment of the affinity to specific drugs

    (e.g. cetuximab, adalimumab, bevacizumab)
- Improbement of diagnostic accuracy

  (e.g. Barrett's esophagus, *C.difficile* infection)

Devices for molecular imaging

- Autofluorescence imaging (AFI)
- Confocal laser endomicroscopy (CLE)
- Endoscopes for wide-field detection?

Different techniques used for CLE in the GI tract

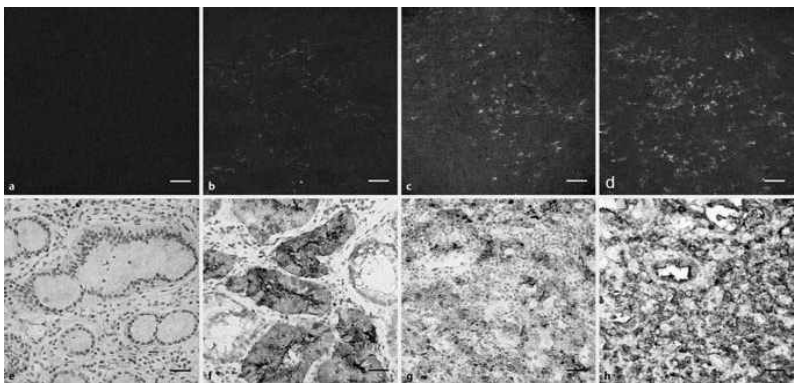
Ref.	Design	No	Target	Composite	Administration	CLE system
Hsiung et al	*In vivo*	26	Colorectal adenomas	Fluorescein-labelled septapeptides	Topical	Probe-based
Foersch et al	Animal, and *ex vivo*	25 and 14	CRC	Alexa-Fluor 488-labelled VEGF-antibodies	Intravenous and topical	Endoscope-based
Goetz et al	Animal and *ex vivo*	68 and 16	CRC	FITC-labelled anti-EGFR-antibodies	Intravenous and topical	Endoscope-based
Foersch et al	Animal and *ex vivo*	12 and 4	CRC	Alexa-Fluor 488-labelled bevacizumab	Intravenous and topical	Endoscope-based
Liu et al	*In vivo*	37	CRC and adenomas	Alexa-Fluor 488-labelled anti-EGFR-antibodies	Topical	Endoscope-based
Cârţână et al	*Ex vivo*	4	CRC	Alexa-Fluor 488-labelled anti-CD31-antibodies	Topical	Endoscope-based
Nakai et al	Animal	2	Healthy esophageal and gastric mucosa	Fluorescein conjugated anti-EGFR-antibodies and anti-survivin-antibodies	Submucosal or topical	Probe-based
Hoetker et al	Animal	26	Gastric cancer	FITC-labelled anti-EGFR1-antibodies or Alexa-Fluor 488-labelled cetuximab	Intravenous	Endoscope-based
Nakai et al	Animal	2	Healthy pancreas	FITC-labelled anti-EGFR1-antibodies or Alexa-Fluor 488-labelled cetuximab	Intravital	Needle-based
Atreya et al	*In vivo*	25	Colonic mucosa in Crohn's disease	FITC-labelled adalimumab	Topical	Endoscope-based
Neumann et al	*Ex vivo*	2	Colonic mucosa in Clostridium difficile associated colitis	Fluorescein-labelled Clostridium difficile specific probe	Topical	Endoscope-based
Li et al	Animal and *ex vivo*	20 and 23	Gastric cancer	Alexa-Fluor 488-labelled anti-MG7-Ag-antibodies	Intracardial and topical	Endoscope-based
Goetz et al	Animal	44	CRC	FITC-labelled anti-EGFR-antibodies or Alexa-Fluor 488-labelled cetuximab	Intravenous	Endoscope-based
Neumann et al	*Ex vivo*	N/A	Barrett's esophagus	FITC-labelled Muc2-antibodies	Topical	N/A
Sturm et al	*In vivo*	25	Barrett's esophagus	FITC-labelled peptides	Topical	Probe-based

Different techniques used for CLE in the GI tract

Design	Target	Target	Administration
Animal (Xenograft tumors) In vivo Ex vivo	Colorectal cancer Colorectal adenomas Gastric cancer Pancreas Crohn's disease C. Difficile colitis Barrett's esophagus	EGRF VEGF Bevacizumab CD31 Cetuximab Adalimumab C.Difficile specific probe MG7-Ag Muc2 Peptides	Intravenous Topical

In vivo molecular imaging of gastric cancer by targeting MG7 antigen with confocal laser endomicroscopy

- Gastric cancer: Specific signals (+ to +++) in 22/23
- Noncancerous: no (0; n=18) or only weak (+; n=5) fluorescent signals



Z.Li et al. Endoscopy 2013;45(02):79-85

MOLECULAR IMAGING OF GASTRIC NEOPLASM BY TARGETING EGFR WITH CONFOCAL LASER ENDOMICROSCOPY: A PILOT STUDY

Background

Tumors of distal esophagus/EGJ/cardia (58.1%)

EGFR expression ↑

- Target therapy
- Cancer-specific survival
- Recurrence

Birkman, et al. BMC Cancer (2016) 16:406

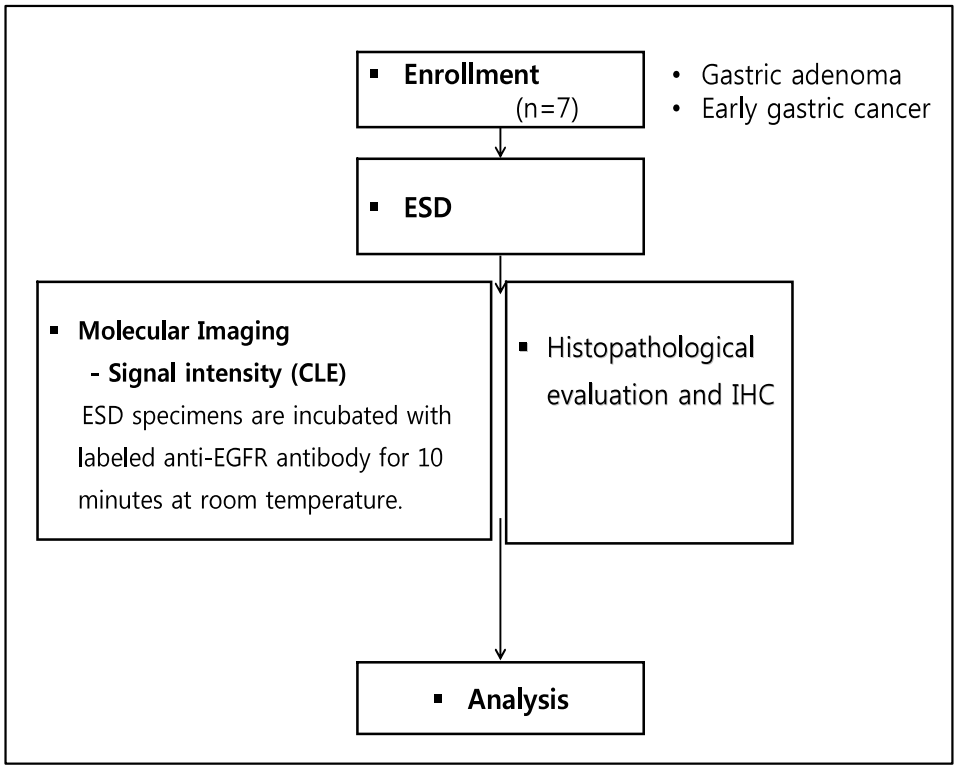
Aim

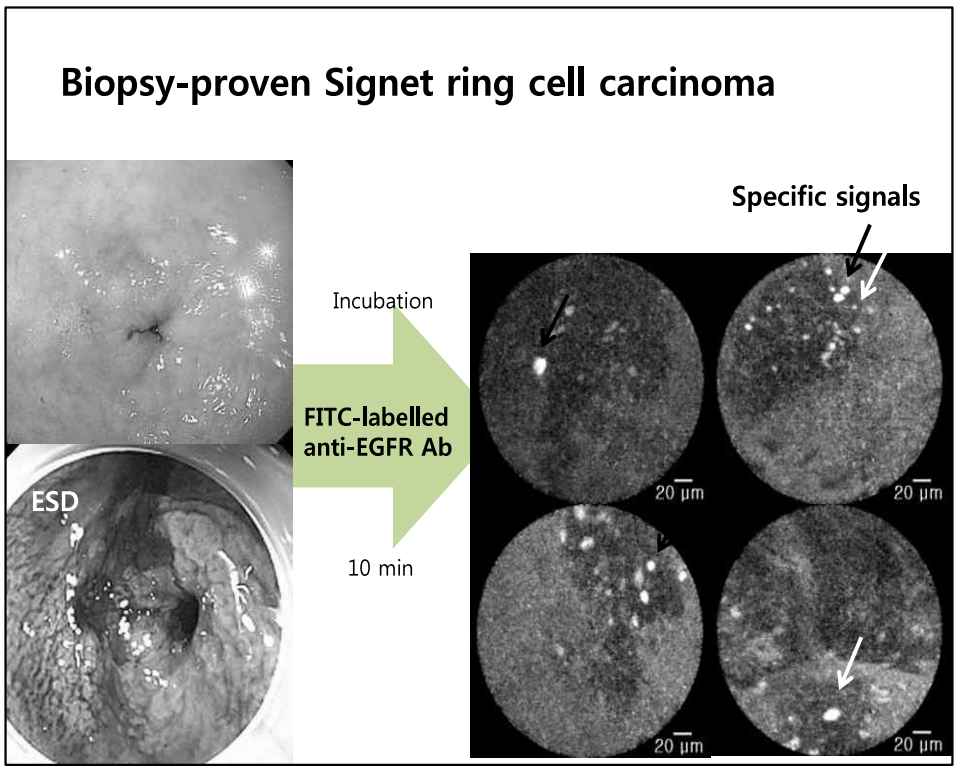
Potential use of EGFR targeted imaging with CLE, after the topical application of labeled antibody ?

We studied on-site assessment of EGFR expression with confocal laser endomicroscopy (CLE) immediately after endoscopic resection of gastric neoplasms.

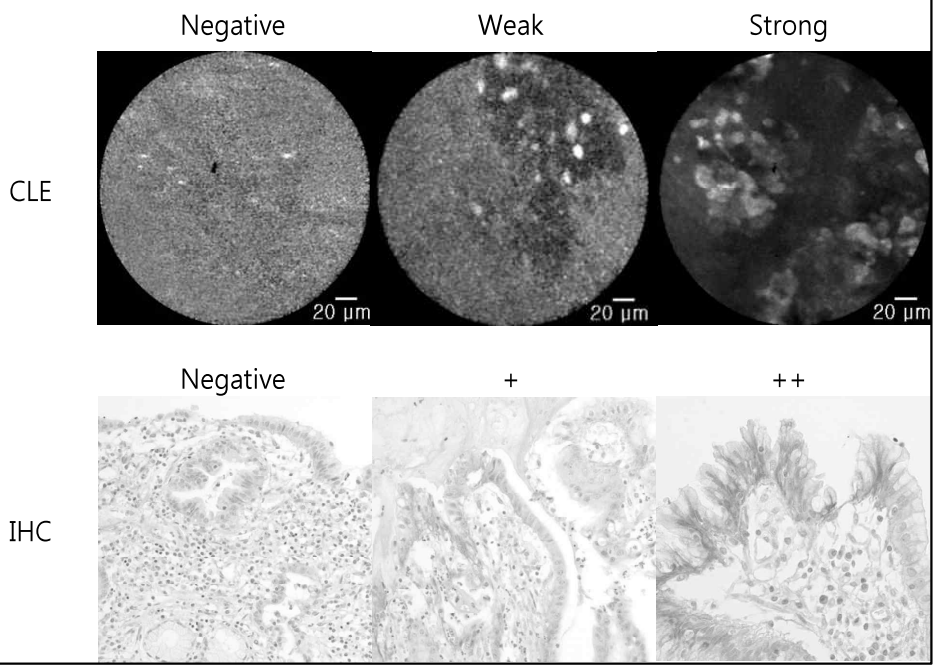
Methods

- Prospective study
- Patients who underwent endoscopic resection (EMR, ESD) for gastric neoplasms
- August 2016-October 2016
- Outcome measures:
  - ✓Signal detection and signal intensity on confocal fluorescent imaging in Gastric neoplasms
  - ✓Degree of agreement between endomicroscopy and IHC results


▪ Enrollment
(n=7)
• Gastric adenoma
• Early gastric cancer
▪ ESD
▪ Molecular Imaging
- Signal intensity (CLE)
ESD specimens are incubated with labeled anti-EGFR antibody for 10 minutes at room temperature.
▪ Histopathological evaluation and IHC
▪ Analysis


Biopsy-proven Signet ring cell carcinoma
ESD
Incubation
FITC-labelled anti-EGFR Ab
10 min
Specific signals
20 μm
20 μm
20 μm
20 μm

Quantification of specific ex vivo fluorescence



Results

Table 1. On-site CLE signal intensity

No.	Sex	Age	Endoscopic Diagnosis	Location	On-site CLE signal intensity
1	F	59	EGC	Prepylorus	Weak
2	F	82	EGC	Antrum	Weak
3	M	53	Adenoma	Prepylorus	-
4	F	66	EGC	Antrum	Strong
5	F	46	EGC	Upper body	-
6	F	70	EGC	Lower body	Strong
7	M	61	EGC	Lower body	-

Table 2. On-site CLE signal and IHC result

No.	Endoscopic Diagnosis	Location	Final pathology	On-site CLE signal intensity	IHC
1	EGC	Prepylorus	Adenocarcinoma, Poorly differentiated	Weak	+
2	EGC	Antrum	Adenocarcinoma, Well differentiated	Weak	++
3	Adenoma	Prepylorus	Low grade dysplasia	-	-
4	EGC	Antrum	Adenocarcinoma, Moderately differentiated	Strong	++
5	EGC	Upper body	Poorly cohesive carcinoma, including SRC	-	-
6	EGC	Lower body	Adenocarcinoma, Well differentiated	Strong	++
7	EGC	Lower body	Reactive atypia	-	++

- Degree of agreement on signal detection between CLE and IHC

  : Good agreement (Kappa=0.696, p=0.053)

- The correlation between different grades of IHC results and semi-quantitative assessment of in EGFR expression using CLE

  : Good agreement (Spearman's r=0.622, p=0.136)

Conclusion

CLE enables on-site evaluation of cell-surface receptor expression in human stomach tissue specimen.

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