# Molecular genetic differences of Barrett esophagus in biopsies

PhD thesis

**Miklós Máté M.D.**

Doctoral School of Clinical Medicine

Semmelweis University

Consultant: Béla Molnár MD, D.Sc.

Official reviewers:
János Banai MD, D.Sc.
Zoltán Máthé MD, Ph.D.

Head of the Final Examination Committee:
Ilona Kovalszky MD, D.Sc.

Members of the Final Examination Committee:
Antal Péter MD, Ph.D.
András Taller MD, Ph.D.

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## Introduction

During the last 2-3 decades the incidence of the esophagus carcinoma revealed a dramatic increase in Western –Europe and in the USA. This is the fifth most frequent tumor in these countries. The increase of the incidence is still unknown. The esophagus carcinoma is fairly treatable in the early stage, however, in late stages the prognosis is extremely bad. That is why the early diagnosis is so important.

The esophagus carcinoma develops from the metaplasia of the cylindric epithelium, which is considered as a consequence of the gastro-esophageal reflux disease (GERD). This cylindrical epithelium metapalasia is called Barrett's metaplasia. In western countries and in Hungary the GERD affects 15-30% of the population, but Barretts metaplasia emerges in only 10-15% of GERD affected patients.

The Barrett's esophagus is considered as a praecancerosis, because the retrospective follow-up indicates the formation of esophagus carcinoma in 0.5-1.5% of the cases.
Endoscopic follow-up, the degree of metaplasia, the multicentric localization, the involvement of the mucosa and submucosa, the detection of metastatic lymph nodes suggest multiple diagnostic problems without definite answer in our days.

Medication beside endoscopic ablation, mucosectomy reduced radical surgery is controversial among surgeons and gastroenterologists.

Unfortunately, in most of cases of Barrett's carcinomas the disease is recognized in late stadium excluding curative treatment, projecting a poor prognosis.
In spite of aggressive treatment strategies the survival statistics doesn't improve in the last 20 years. The 5 years' survival rate of Barrett's carcinoma recognized in early stages is 80-90 %, compared to the survival rates of 10-15 % in late stadium are thought–provoking. That is why early recognition is important.

We still don't know how and why the Barrett's metaplasia conducts in a well-defined morphologic process through an increasing gradient displasia to the emergence of intramucosal adenocarcinoma.
Apart of reflux disease multiple genetic factors are involved in its formation.
The normal cell cycle is damaged. The expression of growth factors augments the caryocinesis. Because of the mutation of tumor suppressors the cells can't enter apoptosis.

With help of molecular genetic research of recent years we are able to study over the morphological aspect the intercellular changes, because the genetic changes precede morphological changes without doubt.

The question is at which moment do those changes occur in cell function that may indicate malignancy and what are the signs of these changes.
For clinicians these signs or indicators can be instructives regarding the treatment.

## Objectives

The cell cycle related protein expression study can reveal important information concerning the comprehension of cell kinetic changes of Barrett's metaplasia.
In our study we wanted to observe if the previously described molecular genetic changes in displasia and adenocarcinoma are present or not in intact, inflammated and metaplastic esophageal biopsies.

We seek the answer if there is any difference in the observed genetic parameters in intact and metaplastic epithelium.
Does the studied metaplastic epithelium already bear plus information regarding the observed parameters, which are not present in intact or esophagitis affected epithelium and which are detectable with immunohistochemical assay (IHC)?

1. Investigation of the changes of two cell cycle genes (PCNA, EGFR), one tumor suppressor gene (p53) and the apoptotic cells in intact (TUNEL method), inflammatory, and metaplastic epithelium during inflammatorical activity.

2. Investigation of immunohistochemical presence of two mucosal antigenes (SIMA, LIMA) in intact, inflammatory, and metaplasic epithelium.

3. Comparison and evaluation of detected genetic changes in immunohistochemical assays with conventional and a special digital microscope developed in our laboratory.

## Methods

The patients involved are without exception from patients of the III. Surgical Department of S.U., my previous workplace.

The pathological evaluation of the specimens was conducted at the I. Pat Dep. Of S.U.
I lead the study at the Cell analysis Lab of the Dept of Internal Medicine. S. U.

All patients signed the official informed consent form beforehand. A rutin esophageal biopsy was taken during gastroscopy.
Conventional Haematoxillin Eosin (HE) process of esophageal biopsy slides was done, and based on pathological diagnosis we selected the patients involved in the study.

30 intact esophagus, 30 esophagitis and 30 Barrett's metaplasia cases were selected.
As a reference 10 intact cardiac biopsies were analyzed.
From the previously chosen 100 slides, we created new slides for Immunohistochemical process.

Signal transmission was performed by Streptavidin-Biotin-Aminoetil-Karbazol (AEC) technique. The slides were covered in Glycerine-Gelatine. The Immunohistochemical transmission of six gene types were analyzed.

We used the TUNEL reaction for the analysis of the apoptosis, and PCNA for caryocinesis to detect the tumor suppressor gene p53, and EGFR was used for growth factor detection.

We analyzed the changes in small bowel mucosal antigene type (SIMA) and big bowel mucosal antigene type (LIMA) due to inflammatorical process in metaplastic epithelium.

The evaluation of the 600 slides were realized by conventional microscope and with assistance of virtual microscope. On all slides 1000-1000 cells were counted and AEC positive cytoblasts were identified.

The evaluation and comparison of conventional and virtual microscocopical findings was performed by statistical analysis software as well.

## Results

**PCNA expression** in intact oesophagus and cardia was significantly lower than in reflux oesophagitis ($p<0,05$) and moderately lower as in Barrett's metaplasia.
Highest rate was found in GERD.

The lowest **p53 expression** was in intact oesophagus and cardia biopsy, and was significantly lower as in Barrett's biopsy ($p<0,05$). The difference between Barrett's and oesophagitis biopsies was minimal.

Regarding the **TUNEL positivity** there is significant difference between intact and Barrett's biopsy. It was lower ($p<0,05$) in Barrett's biopsies. In iflammatorical biopsy it was slightly higher than in Barrett's.

**EGFR expression** was significantly lower in Barrett's biopsy than in intact oesophagus. ($p<0,05$), but there was a slight difference between intact and inflammatorical biopsies.

Regarding the two mucosal antigene types (SIMA) and (LIMA) there is no significant difference either.

**PCNA, EGFR, TUNEL, SIMA. LIMA and p53 expressions in normal oesophageal and cardiac biopsies, compared to GERD and Barrett's oesophagus.**

| Labelling Index ± SD | PCNA | SIMA | LIMA | P53 | Tunnel | EGFR |
|---|---|---|---|---|---|---|
| Healthy | 0.62+0.15*& | 0.65+0.14 | 0.64+0.14 | 0.65+0.14& | 0.56+0.16*& | 0.51+0.16& |
| GERD | 0.83+0,06 * | 0.67+0.14 | 0.67+0.12 | 0.71+0.13 | 0.46+0.18* | 0.52+0.18 |
| Barrett | 0.72+0,12 & | 0.66+0.17 | 0.66+0.11 | 0.72+0.11& | 0.40+0.12& | 0.43+0.12& |
| significance | *& <0,05 | | | & <0,05 | *& <0,05 | & <0,05 |

**Comparison of conventional and virtual microscopic evaluation**

The evaluation of one slide by conventional microscopic method requires 60 to 90 minutes. The digital evaluation requires 25-30 minutes per slide. Regarding the evaluation of slides there was no significant difference between the two methods.

**Comparison of light and virtual microscopy cell counts**

| Light microscopy Parameters | Virtual microscopy (Spearmann $R^2$ |
|---|---|
| )PCNA | 0,98 |
| p53 | 0,94 |
| SIMA | 0,87 |
| LIMA | 0,92 |
| EGFR | 0,82 |
| TUNEL | 0,97 |

## Conclusions

1. The cell cycle related protein expression can provide important informations regarding the comprehension in cell kinetic changes of the reflux esophagitis and Barrett's metaplasia.

2. Based on our analysis those cell related changes (PCNA, EGFR,p53, TUNEL) are already present in esophagitis and Barrett's metaplasia, as in dysplasia adenocarcinoma.

3. The increase of PCNA and p53 expression in Barrett's metaplasia and esophagitis indicates the start up of cell cycle process.

4. The decrease of TUNEL expression in Barrett's metaplasia indicates the changes in apoptosis process compared to intact esophageal mucosa.

5. Decreased EGFR expression in Barrett's metaplasia needs further investigation.

6. Regarding mucosal antigenes (SIMA, LIMA), there is no significant difference between the three analyzed groups, which indicates that the expression of the mucosal antigene production is more expected in displasic mucosa. This can be a prognostical sign.

7. Digital microscopy compared to conventional microscopy gives similar good results, but the analysis is faster, easier and more user friendly.

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