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Epidermal growth factor receptor overexpression/amplification in adenocarcinomas arising in the gastrointestinal tract

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ABSTRACT

Introduction: it has been suggested that EGFR might be valuable to select patients for immunotherapy for various types of cancers.

Aims: we investigated: a) the gene/proteins alterations in gastrointestinal cancers using immunohistochemistry (IHC) (gene overexpression) and fluorescence *in situ* hybridisation (FISH) (gene amplification); and b) the associations between EGFR overexpression and amplification and chromosome 7 aneusomy (CEP7) in these cancers.

Methods: 64 tumor specimens were evaluated by IHC and FISH: 17 adenocarcinoma arising in Barrett's esophagus, 21 stomach cancers, 17 colon cancers, and 9 liver metastasis of colon carcinoma. IHC for EGFR was scored at 4 levels of intensity of membrane staining. EGFR gene in FISH was considered as amplified or not and chromosome 7 (where EGFR is located) as polisomic or disomic. The ratio between EGFR gene and chromosome 7 was performed by FISH and classified the case as gene amplification when the ratio was > 2. Polisomy was identified when the copies of chromosome 7 were > 2 in more than 8% malignant cells.

Results: no difference was found between EGFR gene amplification/protein overexpression according to cancer site. Concerning IHC, most cases were positive for EGFR intensity (84.4%), while only 50% of cases were positive considering a cut-off of 10%. EGFR FISH amplification was found in 4 cases only (6.2%) and FISH CEP7 aneusomy in 40.6%. A statistically significant association was found between EGFR protein positivity (IHC) in term of intensity and EGFR gene amplification by FISH (p = 0.003), and between the EGFR protein positivity (IHC) and chromosome 7 aneusomy (FISH) (p = 0.004).

Conclusions: EGFR amplification assessed by FISH was found in only 4 cases (6.2%) while chromosome 7 aneusomy was identified in 26 (40.6%) cases. IHC proved that EGFR protein overexpression in gastrointestinal cancers is common but FISH assessment showed that EGFR gene amplification is rare. An association was observed between EGFR gene amplification and EGFR protein overexpression in a low number of cases (p = 0.003). A statistically significant association was found between EGFR protein overexpression and chromosome7 polisomy (p = 0.004).

Key words: EGFR. Barrett's esophagus. Colon. Stomach. Liver.

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INTRODUCTION

The epidermal growth factor receptor (EGFR) is a member of the human epidermal growth factor receptors family, which consists of four distinct members: HER1 or EGFR, HER2 (also termed ErbB2 or HER2/neu), HER3 (also termed ErbB3), and HER4 (also termed ErbB4). These receptors share the same molecular structure with an extracellular, cystein-rich ligand-binding domain, a single alphahelix transmembrane domain, and an intracellular domain with tyrosine kinase (TK) activity in the carboxy-terminal tail (except HER3) (1).

Both EGFR and HER-2 receptors are targets for immunotherapy, and for this reason their protein expression and gene amplification are widely investigated. In fact, EGFR has been studied in a variety of pathological conditions such as colorectal cancer, where it was identified as a biomarker (2) and a target for immunotherapy (3), lung cancer (4,5), liver metastasis from colonic carcinomas (6), Barrett's adenocarcinoma (7-9), pancreatic ductal adenocarcinoma (10), gastric cancer (11,12), head and neck squamous cell carcinomas (13) and ovarian carcinomas (14).

Under physiological conditions ligand binding is required to activate EGFR; however, in tumor cells there are additional mechanisms of EGFR activation such as receptor overexpression and autocrine production of ligands by tumor cells (15,16).

EGFR overexpression has been associated with advanced stages of disease, resistance to conventional treatments, and poor prognosis (15,17). The anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies cetuximab and panitumumab seem to have a good clinical activity in about 10% of patients with metastatic colorectal cancer resistant to chemotherapy (3); however, the molecular mechanism underlying clinical response or resistance to these agent are still under investigation. Moreover, a standardized method of measurement and of patients' selection is not universally accepted at the moment.

Some authors have proposed that the response to anti-EGFR treatment in colon and lung carcinomas has a genetic background and suggested to select patients on the basis of EGFR copy number (3,18), while others studies provided evidence that the presence of EGFR mutations –rather than copy number– is more important in determining the outcome with anti-EGFR therapy (4,19).

The aims of the present study were to investigate: a) the gene/proteins alterations of cancers arising in Barrett's esophagus, stomach, colon, and of the liver metastasis of colon cancer, by means of immunohistochemistry (IHC) and fluorescence *in situ* hybridisation (FISH); and b) the association between EGFR overexpression and amplification and chromosome 7 aneusomy in gastrointestinal cancers.

MATERIAL AND METHODS

Pathological evaluation

Immediately after sampling, all specimens were fixed in 10% neutral-buffered formalin for 24 hours, then were included in paraffin and stained with hematoxylin-eosin (H&E) and Alcian-PAS for routine histological examination. H&E-stained slides from the resected specimens were evaluated for identification of the steps in cancer progression. All the carcinomas were diagnosed according to the WHO classification (20).

Sixty-four tumor specimens were evaluated: 17 adenocarcinomas arising in Barrett's esophagus, 21 stomach cancers (6 diffuse type, 14 intestinal type, 1 intestinal type with mucoid differentiation), 17 colon cancers (14 moderately-poorly differentiated adenocarcinomas, 3 mucoid) and 9 liver metastasis of colon carcinoma (2 cases were metastasis of above colon cancer and 7 were from different cases).

Immunohistochemistry

EGFR (HER1) receptor status was analyzed by the EGFR pharmDx kit (DAKOCytomation, Carpinteria, CA, USA). According to the recommendations from the manufacturers, tissue sections mounted on slides and stored at room temperature (25 °C) were stained within 4-6 weeks from sectioning, in order to maintain the antigenicity, and then the samples were counterstained with Mayer's hematoxylin. HER-1 oncoprotein expression was evaluated by two observers, following the score system suggested by the manufacturer's instruction.

Concerning EGFR assessment, this was considered positive when it primarily stained cell membrane, demonstrating both complete and incomplete circumferential staining. The immunostaining pattern was frequently heterogeneous, exhibiting various staining intensities within a single neoplasm. Since there are no guidelines for scoring the samples, in agreement with previous published studies (21,22) we calculated a score based on the stain intensity of tumor cells: 0/1 (no or incomplete membrane staining); 2 (weak/ moderate complete membrane staining); and 3 (strong and complete membrane staining). Moreover, we considered the individual percentage of positive cells in each sample.

FIuorescence *in situ* hybridization (FISH)

EGFR is located on chromosome 7p12 and in FISH it is investigated by a LSI© Locus Specific Identifier DNA Probe labeled by Spectrum Orange fluorochrome (Vysis Inc., Downers Grove, IL, USA). The LSI© probe consists of DNA probe sequences homologous to specific DNA regions. Gene sequences or loci are directly labeled with one of the Vysis fluorophores. Unlabeled blocking DNA is included with the probe to suppress sequences contained within the loci which are common to other chromosomes. When hybridized and visualized, these probes show specific changes, such as amplification, deletion or translocation to specific gene, loci or chromosomal regions. We analyzed also the centromeric region of chromosome 7 (7p11.1-q11.1) with a Chromosome Enumeration Probe (CEP7) labeled by Spectrum Green fluorochrome. The whole area of each neoplastic lesion present in the tissue section was independently evaluated by two investigators (ER, VV) with a fluorescence microscopy (Nikon Optiphot-2) equipped with selective filters for the fluorochromes used, in high power fields (HPF; magnification 600x). FISH images were captured and elaborated using Genikon software (Nikon Instruments S.p.A, Italy). The EGFR gene locus was classified as amplified if there were more than twice the number of red (Spectrum Orange labeling) EGFR signals than green (Spectrum Green labeling) centromere 17 signals (ratio > 2:1) per cell nucleus, as previously described (3). Polisomy was identified when the copies of chromosome 7 were more than 2 in more than 8% of malignant cells.

Reference values for abnormal FISH results were based on criteria of Qian and colleagues for tissue sections, to account for the potential artifacts due to nuclear overlapping in fixed sections. According to these criteria an abnormal autosomal gain required a minimum 8% nucleus with three or more signals, whereas abnormal autosomal loss required more than 55% nuclei with zero or one signal (23).

Table I. Patient's distribution and evaluation

#	Sex	Age	Anatomic site	Diagnosis	EGFR IHC intensity	EGFR IHC %	EGFR FISH	EGFR CEP7	Ratio EGFR/CEP7	Outcome 3 years
1	M	63	Esophagus	ADC all thickness no LMN	1	5	NA	Polisomy	1.2	Alive
2	M	70	Esophagus	ADC all thickness no LMN	0	0	NA	Disomy	1	Alive
3	F	65	Esophagus	ADC all thickness 7 LMN	1	70	NA	Polisomy	1.55	Alive
4	M	56	Esophagus	ADC all thickness no LMN	0	0	NA	Disomy	0.9	Alive
5	M	67	Esophagus	ADC submucosa no LMN	2	65	NA	Polisomy	1.68	NA
6	M	73	Esophagus	ADC all thickness no LMN	3	96	A	Polisomy	5.8	Died
7	M	64	Esophagus	ADC all thickness no LMN	3	80	NA	Disomy	1	Died
8	M	59	Esophagus	ADC all thickness no LMN	3	80	NA	Polisomy	1.9	Alive
9	M	74	Esophagus	ADC all thickness no LMN	1	1	NA	Polisomy	1.2	Alive
10	M	68	Esophagus	ADC submucosa no LMN	0	0	NA	Disomy	1	Alive
11	F	57	Esophagus	ADC all thickness 4 LMN	1	1	NA	Polisomy	1.35	Died
12	M	64	Esophagus	ADC all thickness 2 LMN	3	80	NA	Disomy	1	Died
13	M	69	Esophagus	ADC all thickness no LMN	2	10	NA	Disomy	1	Alive
14	M	63	Esophagus	ADC all thickness no LMN	1	5	NA	Disomy	0.83	Alive
15	M	68	Esophagus	ADC submucosa no LMN	3	10	NA	Disomy	1.01	Alive
16	M	71	Esophagus	ADC submucosa no LMN	0	0	NA	Disomy	0.91	Alive
17	M	60	Esophagus	ADC all thickness 14 LMN	2	20	NA	Disomy	1	Died
18	M	77	Stomach	ADC early antrum	1	10	NA	Disomy	1	Alive
19	M	54	Stomach	ADC all thickness antrum no LNM	1	5	NA	Disomy	1	NA
20	M	58	Stomach	ADC all thickness antrum no LNM	3	20	NA	Polisomy	1.55	NA
21	M	62	Stomach	ADC all thickness corpus no LNM	2	60	NA	Polisomy	1.63	Alive
22	F	66	Stomach	ADC all thickness cardias 3 LNM	2	40	NA	Disomy	0.99	NA
23	F	59	Stomach	ADC all thickness cardias 5 LNM	1	5	NA	Polisomy	1.74	Alive
24	M	69	Stomach	ADC all thickness antrum no LNM	1	40	NA	Disomy	1	Alive
25	F	70	Stomach	ADC all thickness antrum no LNM	3	15	NA	Polisomy	1.68	Alive
26	M	53	Stomach	ADC all thickness corpus 2 LNM	3	10	NA	Disomy	1	Alive
27	M	63	Stomach	ADC all thickness cardias 5 LNM	0	0	NA	Disomy	1	Alive
28	M	66	Stomach	ADC all thickness cardias no LNM	1	2	NA	Disomy	0.99	Alive
29	M	71	Stomach	ADC all thickness antrum no LNM	2	60	NA	Disomy	0.85	Alive
30	M	77	Stomach	ADC all thickness antrum 2 LNM	2	90	NA	Disomy	1	Died
31	M	65	Stomach	ADC all thickness cardiac no LNM	1	5	NA	Disomy	0.87	Alive
32	F	56	Stomach	ADC mucoid all thickness antrum no LNM	3	90	A	Polisomy	4	NA
33	M	63	Stomach	Diffuse type corpus all thickness 5 LNM	1	5	NA	Disomy	1	Alive
34	F	61	Stomach	Diffuse type corpus early no LMN	3	10	NA	Disomy	0.87	Died
35	F	74	Stomach	Diffuse type angulus all thickness 15 LMN	1	30	NA	Disomy	1	Died
36	M	64	Stomach	Diffuse type corpus all thickness 6 LMN	1	5	NA	Disomy	1	Died
37	M	46	Stomach	Diffuse type corpus all thickness 12 LMN	3	50	NA	Disomy	0.89	Died
38	F	42	Stomach	Diffuse type antrum all thickness 5 LMN	0	0	NA	Disomy	1	Alive
39	M	58	Colon	ADC Dukes C	3	90	A	Disomy	2.15	Alive
40	M	67	Colon	ADC Dukes B	3	90	NA	Polisomy	1.98	Alive
41	F	64	Colon	ADC Dukes B	1	1	NA	Polisomy	1.2	Alive
42	M	48	Colon	ADC Dukes C	1	1	NA	Polisomy	1.1	Alive
43	M	69	Colon	ADC Dukes B	1	5	NA	Polisomy	1.5	Alive
44	F	73	Colon	ADC Dukes B	2	5	NA	Disomy	1	Alive
45	F	75	Colon	ADC Dukes C	0	0	NA	Disomy	1	Alive
46	M	64	Colon	ADC Dukes B	1	5	NA	Polisomy	1.25	Alive
47*	M	66	Colon*	ADC* Dukes D	2	65	NA	Disomy	1	Died
48*	M	59	Colon*	ADC* Dukes B	2	70	NA	Disomy	0.9	Alive
49	M	71	Colon	ADC Dukes B	3	80	NA	Disomy	1	Alive
50	M	65	Colon	ADC Dukes C	1	5	NA	Disomy	0.98	Alive
51	M	69	Colon	ADC Dukes C	1	10	NA	Polisomy	1.58	Alive
52	M	57	Colon	ADC Dukes B	3	10	A	Polisomy	12	Alive
53	M	62	Colon	ADC mucoid Dukes B	1	5	NA	Polisomy	1.5	Alive
54	F	67	Colon	ADC mucoid Dukes B	2	80	NA	Polisomy	1.3	Alive
55	M	72	Colon	ADC mucoid Dukes C	0	0	NA	Disomy	0.88	Alive
56	M	71	Liver	Metastasis of colon ADC	1	1	NA	Polisomy	1.45	Died**
57	M	67	Liver	Metastasis of colon ADC	0	0	NA	Disomy	1	NA
58	F	58	Liver	Metastasis of colon ADC	1	1	NA	Polisomy	1.5	NA
59	M	48	Liver	Metastasis of colon ADC	1	1	NA	Polisomy	1.25	Died
60	M	64	Liver	Metastasis of colon ADC	1	1	NA	Polisomy	1.2	Alive**
61	M	66	Liver	Metastasis of colon ADC	0	0	NA	Disomy	0.9	NA
62	M	75	Liver	Metastasis of colon ADC	1	1	NA	Polisomy	1.8	NA
63*	M	66	Liver*	Metastasis of colon ADC*	1	10	NA	Disomy	1	Died
64*	M	59	Liver*	Metastasis of colon ADC*	2	10	NA	Disomy	0.9	Alive**

F: female; M: male; ADC: adenocarcinoma; FISH: fluorescence *in situ* hybridization; IHC: immunohistochemistry; A: amplified; NA: not amplified; CEP7: chromosome enumeration probe for chromosome 7. *The cases analysed for the ADC in colon and its liver metastasis. **Treated with epidermal growth-factor receptor (EGFR) inhibitors cetuximab (*Erbitux*).

Table II. EGFR protein IHC intensity, EGFR protein IHC percentage, EGFR gene FISH evaluation, chromosome 7 (CEP7) FISH evaluation according to anatomic site

	Anatomic site				
	Esophagus cancer	*Stomach cancer*	*Colon cancer*	*Liver metastasis of colon ADC*	*Total*
EGFR IHC intensity	No. (%)	No. (%)	No. (%)	No. (%)	
0	4 (23.5)	2 (9.5)	2 (11.8)	2 (22.2)	10 (15.6)
1	5 (29.4)	9 (42.8)	7 (41.2)	6 (66.7)	27 (42.2)
2	3 (17.7)	4 (19.1)	4 (23.5)	1 (11.1)	12 (18.9)
3	5 (29.4)	6 (28.6)	4 (23.5)	0 (0)	15 (23.4)
EGFR %					
≥ 10%	9 (52.9)	13 (61.9)	8 (47.1)	2 (22.2)	32 (50)
< 10%	8 (47.1)	8 (38.1)	9 (52.9)	7 (77.8)	32 (50)
EGFR FISH					
A	1 (5.9)	1 (4.8)	2 (11.8)	0	4 (6.3)
NA	16 (94.1)	20 (95.2)	15 (88.2)	9 (100)	60 (93.7)
CEP7 FISH					
Polisomy	7 (41.2)	5 (23.8)	9 (52.9)	5 (55.6)	26 (40.6)
Disomy	10 (58.8)	16 (76.2)	8 (47.1)	4 (44.4)	38 (59.4)
Total	17 (100)	21 (100)	17 (100)	9 (100)	64 (100)

FISH: fluorescence *in situ* hybridization; IHC: immunohistochemistry; A: amplified; NA: not amplified; CEP: chromosome enumeration probe.

Applying the same criteria used in the HER-2/neu evaluation (24,25), the cell population of each HPF was classified as displaying a disomy, an aneusomy (generally a polisomy) or a gene amplification.

Statistical analysis

IHC was scored as 0,1,2, and 3 depending on intensity of membrane staining, and categorized at 4 levels while the number of positive cells were indicated in percentage and dicotomized in < 10 (negative) and ≥ 10 (positive). FISH for EGFR gene was considered positive when amplified and negative when not amplified. FISH for chromosome 7 was considered positive in presence of chromosome aneusomy (polisomy) and negative in presence of chromosome disomy.

The associations between EGFR protein intensity in IHC, EGFR protein percentage of positivity in IHC, EGFR gene in FISH, chromosome 7 aneusomy in FISH and the cancer site, and the associations between EGFR gene amplification and protein overexpression and chromosome 7 polisomy were evaluated using the usual statistical methods for comparison of proportions. p-values lower than 0.05 (two-tailed tests) were used to reject the null hypothesis.

RESULTS

Overall, specimens from 64 patients were obtained. The demographic characteristics, cancer anatomic site, histological diagnosis, and the results of EGFR gene amplification, protein overexpression and the ratio EGFR/CEP7 are shown in table I. The mean age was 67.9 with a range of 34-91 (SD: 11.7) years; most patients (78.1%) were males. Patients with esophagus and stomach cancers were older (mean ages: 73.1 and 70.4 years, respectively) than those with colon cancer (mean age: 63.9 years) and with liver metastasis (mean age: 59.4 years) (p = 0.008).

Most cases resulted positive when evaluating intensity for EGFR by IHC (n = 54, 84.4%); in fact, only 10 cases (15.8%) showed score 0, whereas by considering the percentage of positive cells ≥ 10%, 50% of cases resulted positive (Table II).

EGFR amplification visualized by FISH was found in only 4 cases (6.2%) and chromosome 7 aneusomy was identified in 26 (40.6%) cases.

The distribution of gene amplification/protein overexpression/chromosome 7 aneusomy (polisomy) according to cancer site are shown in table II. No statistically significant difference was found in EGFR overexpression/amplification and chromosome 7 polisomy according to cancer sites.

Representative images showing FISH and IHC for EGFR in various cancer sites and histological subytpes are shown in figures 1-3.

As summarised in table III, a statistically significant association (p = 0.003) was found between EGFR protein intensity score (3+) visualized by IHC and EGFR gene amplification visualized by FISH.

Moreover, a statistically significant association was found between EGFR protein overexpression and chromosome 7 polisomy (p = 0.004).

Table III shows the association between EGFR gene amplification by FISH and the number of positive cells visualized by IHC; though not statistically significant, all

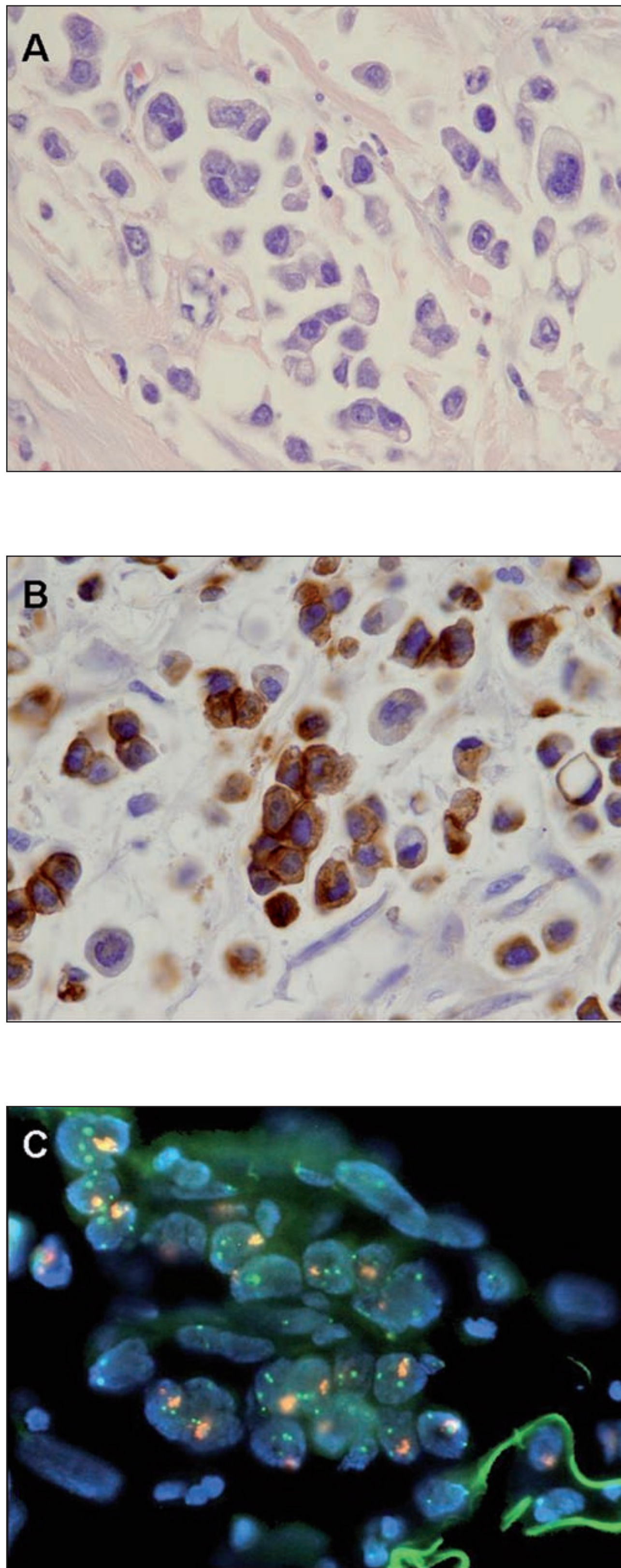

Fig. 1. Stomach cancer (case #32, table I) A. Hematoxylin and eosin stain. B. IHC for EGFR shows a protein overexpression (brown stain). C. FISH for EGFR (red spots) and chromosome 7 (green spots) shows a strong gene amplification and clustering.

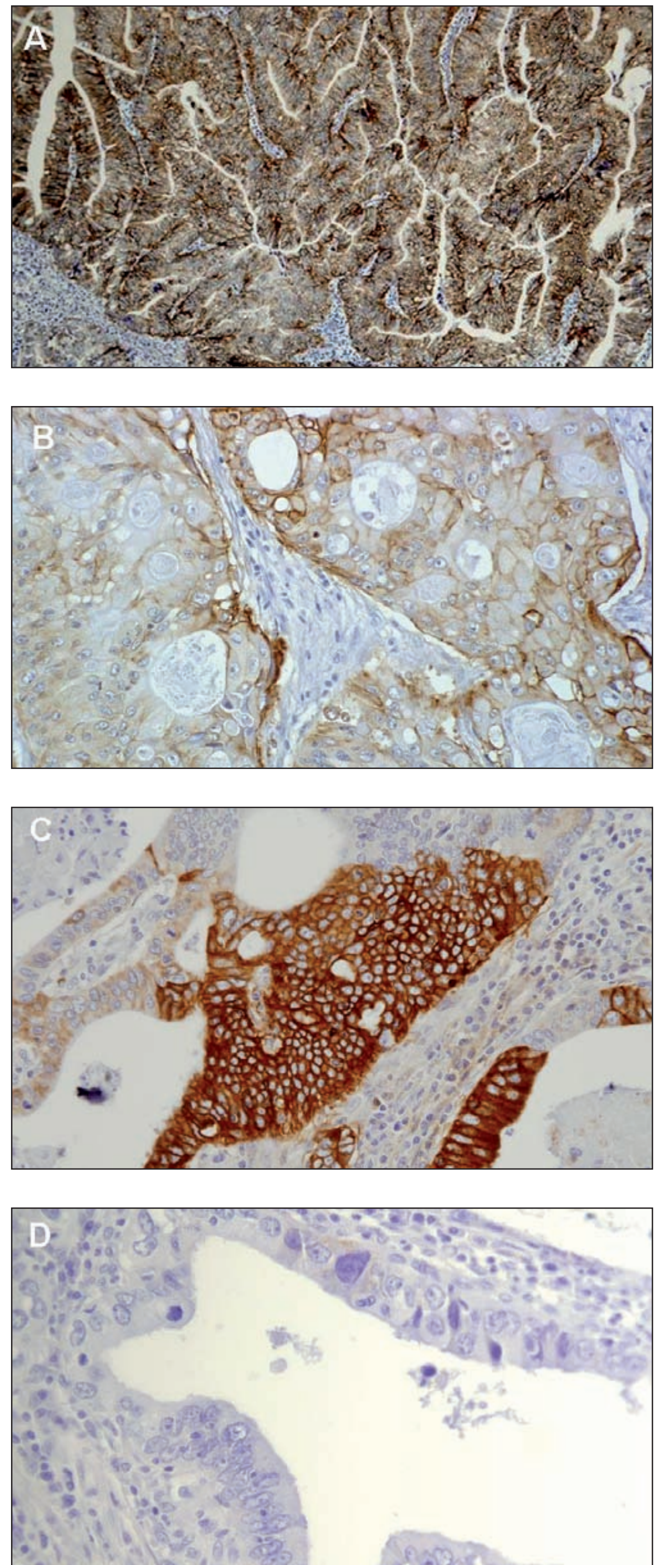

Fig. 2. IHC for EGFR. A. ADC arised in BE (case #12, table I) show a strong protein overexpression (3+). B. Adenocarcinoma moderately differentiated of the colon (case #40, table I) scored 2 + as intensity in IHC evaluation of EGFR. C. ADC in stomach cancer scored 3 + in the central area which was only the 15% of the tumpur (case #25, table I). D. Negative (0) stomach ADC (case #38, table I).

4 cases amplified by FISH showed ≥ 10% of malignant cells by IHC positivity. No significant association was found between EGFR IHC % positivity and chromosome 7 aneusomy. Three out 4 cases amplified by FISH (75%) *vs*. 23 out 60 (38.3%) not amplified had chromosome7 polisomy ($p > 0.05$) (data not shown).

DISCUSSION

Anticancer drug discovery has shifted from an empiric random screening approach to a more rational, target-directed approach. The use of small molecules with tyrosine kinase inhibitory activity directed toward the EGFR, such as gefitinib for non-small cell lung cancer (NSCLC) or erlotinib for NSCLC and pancreas cancer, represent interesting examples. However, these therapies have modest activity when given to unselected patient populations.

One aim of this study was to investigate the EGFR gene amplification/protein overexpression in gastrointestinal tract cancer using both FISH and IHC techniques. We found that most cases were positive for EGFR by IHC (54 out 64, 84.4%), whereas very few (4 out 64, 6.3%) showed EGFR gene amplification.

We did not find any differences in the prevalence of EGFR amplification/overexpression according to different cancer sites and types, although the small number of patients may be a limiting factor in our study. It is however worth noting the rarity of the EGFR gene amplification, observed in 1 out 17 cases of ADC arising in BE, 1 out 21 cases of mucoid ADC of the stomach, and in 2 out 17 cases of colon ADC. No liver metastasis of colon ADC were found positive by IHC or FISH for EGFR, and this could be due to number of the metastases analyzed or to a different behaviour of metastasis (cases # 63 and 64) compared to primitive cancer (cases # 47 and 48).

It is well known how in other pathologies the absence of gene amplification in cases which display a protein overexpression could be due to a polisomy of the chromosome where the gene is located. This is true, for example, in breast cancer referred to HER-2 evaluation (26) where polisomy of chromosome 17 plays an important rule.

We investigated the possible association between EGFR and chromosome 7 polisomy, which is considered a marker of tumor progression, often present in carcinomas (27). Not surprisingly, we found an association between chromosome 7 and EGFR gene overexpression evaluated by IHC intensity. The association between chromosome 7 aneusomy and EGFR percentage of positive cells by IHC was not statistically significant due to the small number of amplified cases.

According to the DakoCytomation EGFR pharmDX kit for EGFR testing a positive IHC stain for EGFR is defined as ≥ 1% of tumor cells showing partial or circumferential membrane staining of any intensity (above background), but many authors suggest to consider the membrane intensity with a score (0,1,2,3) and the percentage of positive cells (21,22). We decided to consider these parameters separately and to investigate the possible associations between

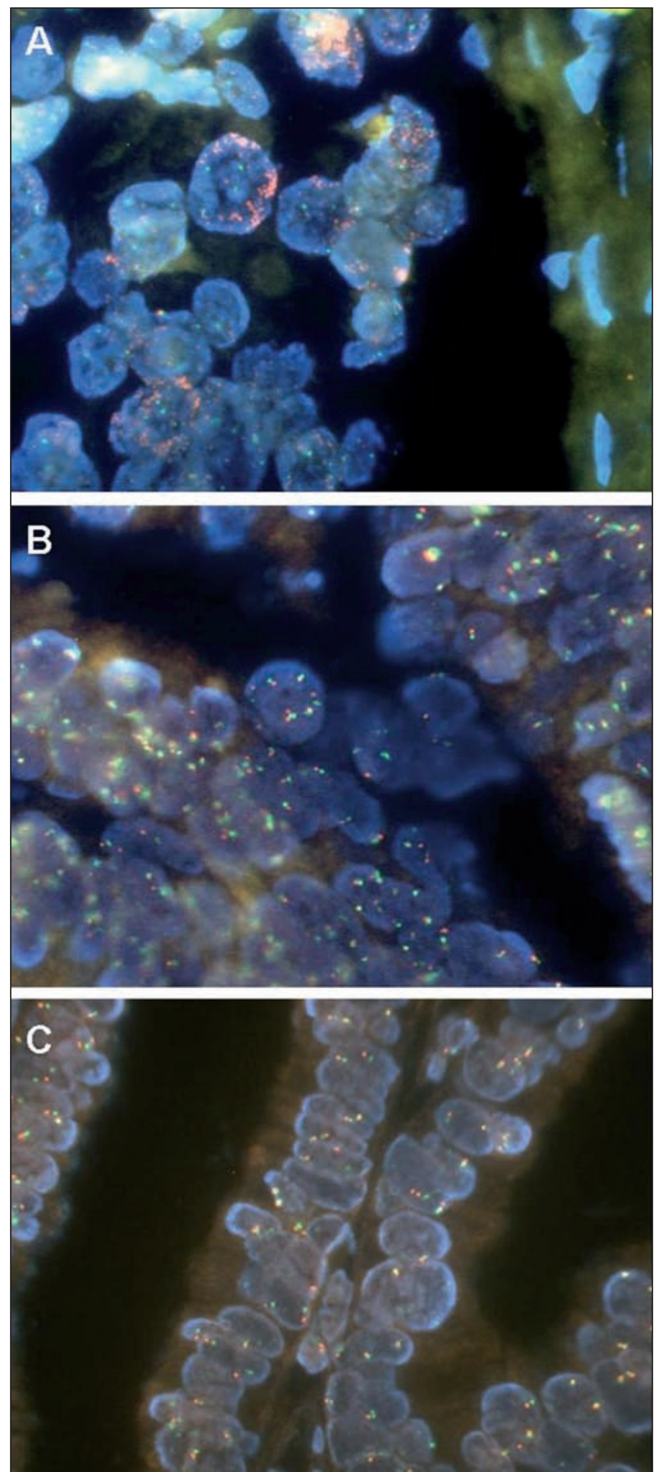

Fig. 3. FISH for EGFR. A. Gene amplification in colon cancer (case #52, table I) as show the red spots (EGFR gene) spread in the nucleus. B. Polisomy for chromosome 7 (CEP7 green spots) in colon ADC (case #40, table I). C. EGFR not amplified and without polisomy for chromosome 7 in ADC arising in BE (case #13, table I).

Table III. Relationship between IHC for EGFR intensity and FISH for EGFR gene and chromosome 7 and relationship between IHC for EGFR positivity (%) and FISH for EGFR gene and chromosome 7

	EGFR IHC intensity score					
EGFR FISH	*0 No. (%)*	*1 No. (%)*	*2 No. (%)*	*3 No. (%)*	*Total No. (%)*	*p value**
Amplified No. (%)	0	0	0	4 (100)	4 (100)	
Not amplified No. (%)	10 (16.7)	27 (45.0)	12 (20.0)	11 (18.3)	60 (100)	
Total	10	27	12	15	64	0.003
FISH CEP7						
Disomy No. (%)	10 (26.3)	11 (28.9)	9 (23.7)	8 (21.1)	38 (100)	
Polisomy No. (%)	0	16 (61.6)	3 (11.5)	7 (26.9)	26 (100)	
Total	10	27	12	15	64	0.004

	EGFR IHC %			
EGFR FISH	*≥ 10%*	*<10%*	*Total No. (%)*	*p value**
Amplified No. (%)	4 (100)	0	4	
Not amplified No. (%)	28 (46.7)	32 (53.3)	60	
Total	32	32	64	NS
CEP7				
Polisomy	12 (46.2)	14 (53.8)	26	
Disomy	20 (52.6)	18 (47.4)	38	
Total	32	32	64	NS

*Exact test ; NS: p > 0.05.

them. In our experience cases with only 1% cell positivity were not found. For this reason we decided to discriminate as follows: positive > 10% and negative < 10%. Thus, most cases resulted positive when evaluating intensity for EGFR by IHC (n = 54, 84.4%), but if we consider the percentage of positive cells ≥ 10% only 50% of cases resulted positive (Table II), which would reduce dramatically the potential patients suitable for therapy.

Among the cases amplified by FISH, though very few (4 out 64), all of them showed the highest intensity by IHC and more than 10% positive cells in EGFR IHC, and 3 of them (75%) showed polisomy of chromosome 7.

Previous studies based on EGFR mutation, amplification, overexpression showed that this gene modifies its behavior depending on the tumors analyzed (3,4) and for this reason there is no convincing and practical way to select patients for the immunotherapy. Moreover, some authors demonstrated that mutation of the KRAS oncogene is a powerful negative predictive biomarker to identify patients with metastatic colon cancer who do not benefit from EGFR-I therapy (28). On the other hand, other authors (3) argued that the confirmation of EGFR overexpression by evaluating EGFR gene amplification by FISH may be important to select patients for colon cancer treatment.

Some patients with high levels of EGFR expression are refractory to EGFR inhibitor treatment, suggesting that mere expression of EGFR is not a robust predictor of response to therapy (29). The lack of a clear relationship between the level of EGFR expression and the degree of EGFR activation across tumor types complicates simple prediction of clinical effectiveness of targeted therapeutic approaches (30).

In conclusion, our study provides further data to the debate regarding the evaluation of EGFR in the GI tract. Even though we found a strong association between EGFR gene/protein expression and chromosome 7 polisomy these expressions seem to characterize only a small percentage of GI carcinomas. This is the first report where GI tract pathology has been analyzed with these techniques at the same time within the different areas where the ADC arose (esophagus, stomach, colon and liver metastasis). The relatively low number of cases is due to the selection ADC arising in the GI tract while avoiding the cases which showed only dysplasia or dysplasia in the majority of the tissue; thus, confirmation of these findings in a larger population could be intriguing.

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