Prognostic and predictive role of EGFR protein expression in colorectal cancer

PhD thesis

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Introduction

Among the countries of the European Union, the standardised colorectal cancer (CRC) mortality rate is the highest in Hungary. The epidermal growth factor receptor (EGFR) is a tyrosine kinase transmembrane receptor, one of the key factors of gene transcription and cell proliferation leading to the progression of the disease.

Metastases occur in 20-50% of all CRC cases during the course of the disease. In unresectable metastatic CRC, systemic therapy is the preferred therapeutic option. The chemotherapy backbone should be combined with targeted agents. Bevacizumab/Avastin is a vascular endothelial growth factor (VEGF) inhibitor. Cetuximab and panitumumab are anti-EGFR monoclonal antibodies. Unfortunately, to date only negative predictive molecular pathological factors are known, such as RAS and BRAF mutations. In daily practice, approximately 40% of patients are

expected to be non-responsive to anti-EGFR antibody therapy. Therefore, it would be useful to have positive predictive markers as well.

The localisation of the primary tumor as a potential prognostic and/or predictive marker has recently received greater attention. It has been confirmed that right-sided tumors have a worse prognosis compared to left-sided CRCs.

In colorectal cancer, EGFR protein expression of the tumor cells was found to be a powerful predictor of prognosis. The predictive value of EGFR protein expression for EGFR antibody treatment is still questionable. Interestingly, there are even reports of the effectiveness of anti-EGFR agents in EGFR-negative cases.

The diagnostic problems of EGFR expression and the observation that EGFR-negative colorectal cancer patients responded to EGFR-targeted antibody therapy have diminshed the use of EGFR IHC in colorectal cancer.

Data on the correlation between EGFR-CNV and the response to anti-EGFR antibody therapy are also still controversional.

There are available data on the correlation between sidedness and EGFR expression of colorectal tumors. EGFR expression is more frequent in right-sided tumors compared to left- sided CRCs. Data also demonstrated that EGFR protein expression was significantly higher in right-sided tumors than in left-sided and rectal tumors.

Aims

1.Evaluate the difference between the EGFR expression of the left- and right-sided tumors in a KRAS exon2 wild -type metastatic colorectal cancer cohort of patients, treated with anti-EGFR therapies, where survival data (PFS, OS) were available.

2. In a small proportion of these patients we have compared the EGFR copy numbers (CN) in tumor cells with their corresponding EGFR protein scores, and investigated the potential correlation.

3. Evaluate the predictive role of EGFR protein expression in the efficacy of anti-EGFR antibody (cetuximab) therapy, and also test the predictive value of the EGFR H-score of primary tumors and metastases in a multivariate analysis.

Methods

We collected data on 99 patients who were diagnosed with metastatic colorectal cancer and treated with anti-EGFR antibody therapy. In total, 97 primary tumors and 33 corresponding metastatic tissues were available for further evaluation. In 31 cases, we had samples from both the primaries and the corresponding metastases for comparison.

EGFR protein expression

The EGFR protein expression of colorectal cancer tumor cells was determined by immuno-histochemistry. The evaluation was carried out applying the H-score (0–300) semiquantitative methodology.

RAS testing

KRAS exon2 mutations were identified by microcapillary-based restriction fragment length analysis. Mutation positivity was defined as samples containing >5% of the non- wild type (WT) band. After protocol changes, extended RAS mutation analysis was done.

Evaluation of EGFR gene copy number using interphase fluorescence in situ hybridization (iFISH)

The EGFR gene copy number status was evaluated by iFISH analysis. Average EGFR copy number/cell, average CEN7 copy number/cell, EGFR/CEN7 ratio, average EGFR copy number/cell in amplified cell population, and percentage of polysomic or amplified cells were calculated.

Statistical analysis

The investigated patient cohort was divided into low and high expression groups based on their EGFR H-scores. We used different EGFR-HS threshold

ranges (0, 50, 100, 200) to define low/high groups. The H-score of EGFR was analyzed by the Mann-Whitney test. Overall and progression-free survival analyses were carried out using the Kaplan–Meier method. The comparison between survival data of different strata was assessed by log-rank statistics. A multivariate analysis was performed by the Cox proportional hazard model.

Results

1. Difference between the EGFR expression of left- and right-sided tumors

We evaluated and compared the EGFR-H-scores of left- (LSCRC) and right-sided colorectal cancers (RSCRC). In primary tumors, we found that the EGFR H-scores of the LSCRC were significantly lower than those of RSCRC: 89.9 ± 66.7 versus 141 ± 72.2 ($p = 0.04$). In metastases, a similar comparison of the right-sided and the left-sided samples was executed. According to our results, EGFR scores of the left-sided CRC metastases were

significantly lower than those of the right sided samples: 86.6 ± 65.2 versus 142.5 ± 87.8 (p=0.018). In addition, progression free survival (PFS) and overall survival (OS) data of anti-EGFR antibody treated RSCRC and LSCRC patients (N= 22 v.75) were investigated using Kaplan-Meier analysis. Regarding PFS, RSCRC patients showed numerically poorer survival compared to the LSCRC cohort (p = 0.064). The difference in OS was significantly worse in RSCRC patients than those with LSCRC (p = 0.047). The median PFS was 189 days for LSCRC and 117 days for RSCRC patients. The median OS for LSCRC was 423 days vs. 265 days for RSCRC.

2. Correlation between the EGFR copy number (CN) and corresponding EGFR protein score

We compared the EGFR copy numbers (CN) in tumor cells to their corresponding EGFR protein scores in 7 cases. We found that in these selected cases CN/cell varied between 1.9 (diploid) and 5.04

(amplified), and the EGFR H-scores varied between 5 and 250. However, there was no association between the CN and EGFR protein expression in these cases. Moreover, extremely low protein scores were associated with amplified tumors, and high scores with near diploid statuses.

3. Predictive role of EGFR protein expression in the efficacy of anti-EGFR antibody (cetuximab) therapy

We analysed the correlation between the EGFR-HS and the progression-free survival (PFS) and overall survival (OS) in the cetuximab FOLFIRI treated group of our patient population (N=90).

We evaluated the EGFR protein expression of the primaries and their metastases. The median EGFR-HS was similar in both the primary and the metastatic tumor tissues (100 ± 66 versus 110 ± 75, respectively). Distribution of the EGFR-H-scores (by 50 increments) were very similar in the primary and the metastatic colorectal tumors.

Comparison of the HS of 27 metastases to their corresponding primaries found significant differences and extreme alterations in both directions (higher or lower) in the majority of cases. The metastases maintained the EGFR-HS range of the primary tumor only in a minority of cases (no difference: 3/27, 11.1%; ±10% difference: 8/27, 29.6%).

EGFR H-scores of the primary tumors with different metastatic potentials (single versus multiple metastatic diseases) were also compared. The EGFR protein expression was significantly higher in primary tumors with multiple metastases (p = 0.007). We evaluated the possible correlation between the metastatic potential and the EGFR expression of the tumors. Our data revealed that both in primary tumors and their metastases, the tissue samples of multiple metastatic cases express significantly higher EGFR-HS compared to the

EGFR expression of samples of single metastatic cases (p = 0.007 and p = 0.004 respectively)**.**

Our data indicated that in primary tumors with values below the threshold, EGFR protein expression was associated with favourable PFS and OS. The differences were statistically significant in OS at the 200 threshold exclusively (p <0.05). In metastatic tissues, our data indicated that values below the applied threshold of EGFR-HS were associated with longer PFS. The differences were significant at the 50 and 200 thresholds in PFS, and at all thresholds in OS. In particular, the difference was greatest at the lowest thresholds, gradually decreasing with increasing EGFR-HS thresholds.

The predictive power of EGFR H-scores of primary tumors and their metastases was also investigated. We applied the Cox proportional hazard model and tested the EGFR-H score in multivariate analysis, with other factors such as sidedness, number of involved metastatic organs (single versus multiple),

age and sex. The analysis confirmed that in our cetuximab treated cohort, EGFR H-score was a very weak independent predictor of OS; it approached the border of significance only in the case of metastatic tissue. In the same analysis, sidedness was found to be a strong, significant predictor either in the group of primary tumors or in metastases. It should be noted that there was a significant difference between the EGFR expression of left- and right-sided CRCs. Finally, we conducted a subgroup analysis of survival data of the left- and right-sided cetuximab treated cases based on EGFR-HS, with low- versus high status determined by the median of the analysed subgroup. In left- or right-sided primary tumors, there was no statistical difference observed in OS between EGFR-low and EGFR-high tumor cases. In case of metastases, the Kaplan–Meier analysis demonstrated that low EGFR-HS patients are characterized by a nominally better median OS at both sides (left side low: 766.5 days versus high: 368

days; right side low: 283.5 days versus high: 55 days). This result was significant only in the case of left-sided tumors (N = 18, $p = 0.016$).

New observations

Our results support the findings that metastatic KRAS-wt LSCRC respond significantly better to anti-EGFR antibody therapy than RSCRC.

Our data also confirmed that RSCRC has a significantly higher EGFR protein expression level than LSCRC, even in KRAS-wt settings. Based on our results, there is no correlation between EGFR CNV and protein expression in CRC.

Our data confirmed that in a cetuximab treated group of KRAS exon2 wild-type metastatic colorectal cancer patients, low EGFR protein expression levels of tumor tissue are associated with significantly better survival.

The multivariate analysis indicated that EGFR protein expression of both the primary as well as the

metastatic tissues is not an independent predictor of cetuximab efficacy.

Based on our results, sidedness and EGFR expression are closely related: the poorly responding right-sided tumors express EGFR at significantly higher levels compared to left-sided CRCs.

Bibliography of candidate's publications

Publications related to the thesis

Uhlyarik A, Piurko V, Vizkeleti L, Pápai Z, Rásó E, Lahm E, Kiss E, Sikter M, Vachaja J, Kenessey I, Tímár J.
EGFR Protein Expression of KRAS Wild-Type Colorectal Cancer: Predictive Value of the Sidedness for Efficacy of Anti-EGFR Therapy.
Pathol Oncol Res. 2020 26(3):1429-1434.
IF: 3,201

Uhlyarik A, Piurko V, Papai Z, Raso E, Lahm E, Kiss E, Sikter M, Vachaja J, Kenessey I, Timar J.
EGFR Protein Expression in KRAS Wild-Type Metastatic Colorectal Cancer Is Another Negative Predictive Factor of the Cetuximab Therapy.
Cancers 2020 12(3):614.
IF: 6,639

Uhlyarik A, Piurkó V, Pápai Z, Rásó E, Lahm E, Kiss E, Sikter M, Vachaja J, Vízkeleti L, Kenessey I, Tímár J.

EGFR protein is a negative prognostic and predictive factor in wild-type RAS colorectal cancer.

Magy Onkol. 2021 65:121–12

Publications not related to the thesis

Kovács M, Németh A, Pák P, **Uhlyarik A**, Pák G, Rácz I.

A kapszulás endoszkópia diagnosztikus értékének és klinikai kihatásának vizsgálata tisztázatlan eredetű gasztrointesztinális vérzésekben.

Orv Hetil. 2006 147: 38 pp. 1827-1833.

Kovács M, Pák P, **Uhlyarik A,** Pák G, Török A, Gervain J, Fehér J.

Vékonybél-stromatumorok diagnózisa kapszulás endoszkópiával.

Orv Hetil. 2008 149: 15 pp. 697-701.

Sikter M, Lahm E, **Uhlyarik A**, Pápai Zs, Ender F.
Centrális pontin myelinolysis.
Magyar Orvos 2009 17: 10 pp. 34-35.

Uhlyarik A, Riedl E, Ágoston P, Sarkadi G, Pápai Zs.
Fulvesztrantkezelés időskorban: hossszú túlélés, jó életminőség.
Lege Artis Medicinae 2011 21: 4 pp. 299-300.

Uhlyarik A , Pápai ZS.
A neuroendokrin daganatok kórisméje és kezelésének irányelvei.
Orv Hetil. 2013 154: 39 pp. 1549-1555.

Uhlyarik A, Lahm E, Vachaja J, Pápai Zs.
Carcinoid szindrómával társuló metasztatikus középbél-eredetű neuroendokrin tumor kezelése.

Orv Hetil. 2014 155: 5 pp. 194-198.

Uhlyarik A, Petrányi Á, Rácz K, Bodoky Gy.
A pajzsmirigyrák gyógyszeres kezelése.
Klin Onkol. 2015 2: 2 pp. 87-92.

Petrányi Á, **Uhlyarik A**, Rácz K, Bodoky Gy.
A gastro-entero-pancreaticus neuroendokrin daganatok onkológiai kezelési lehetőségei.
Klin Onkol. 2015 2: 1 pp. 31-38.

Dede K, Papp G, Salamon F, **Uhlyarik A**, Bursics A.
A máj VII-es szegmentumában igazolt áttéti daganat laparoszkópos reszekciója.
Orv Hetil. 2016 157: 20 pp. 796-800.
IF: 0.349

Uhlyarik A, Lohinszky J, Laki A, Varga Zs, Dabasi G, Tóth M, Igaz P.

A GEP-NET tumorok kezelésével kapcsolatos gyakorlati kérdések, a terápiamegválasztás szempontjai.
Orvostovábbképző Szemle 2018 25: 1. különszám pp. 2-6.

Kocsis J, Szekanecz É, Bassam A, **Uhlyarik A**, Pápai Z, Rubovszky G, Mezősi E, Rucz K, Garai I, Nagy E, Uray I, Horváth Z.
First Line Sorafenib Treatment for Metastatic Medullary Thyroid Cancer: Efficacy and Safety Analysis.
Exp Clin Endocrinol Diabetes. 2019 127(4):240-246.
IF: 2,058

Uhlyarik A.
Az örökletes vastagbélrákok kezelése.
Magy Onkol. 2020 64: 1 pp. 32-37.

Herold Z, **Uhlyarik A**, Herold M, Nagy P, Huszty GD, Rosta K, Doleschall M, Somogyi A.
Regular chromogranin A monitoring facilitated the early detection of a gastrointestinal neuroendocrine tumour in a patient with type 1 diabetes.
Endokrynol Pol. 2020 71(5):483-484.
IF: 1,582

Uhlyarik A.
Agnosztikus terápia onkológiai alkalmazása.
Klin Onkol. 2020 7(4):315–322

Uhlyarik A, Igaz P.
Malignant Paraganglioma .In: Igaz P. *Practical Clinical Endocrinology.* Cham, Svájc: Springer International Publishing *2021 533 p. pp. 383-388.*

Lohinszky J, **Uhlyarik A.**
Humoral Hypercalcemia of Malignancy. In: Igaz P. *Practical Clinical Endocrinology*. Cham,

Svájc: Springer International Publishing *2021 533 p. pp. 481-487.*