Radiotherapy and Oncology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original article

High tumor glycine concentration is an adverse prognostic factor in locally advanced rectal cancer

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ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form 23 November 2015
Accepted 28 November 2015
Available online xxxx

Keywords:
Glycine
Metabolism
Metastasis
Radiotherapy
Rectal cancer
Survival

ABSTRACT

Background and purpose: Recognizing the link between altered tumor metabolism and disease aggressiveness, this study aimed to identify associations between tumor metabolic profiles and therapeutic outcome in locally advanced rectal cancer (LARC).

Materials and methods: Pretreatment tumor metabolic profiles from 54 LARC patients receiving combined-modality neoadjuvant treatment and surgery were acquired by high-resolution magic angle spinning magnetic resonance spectroscopy (HR MAS MRS). Metabolite concentrations were correlated to TNM and the presence of disseminated tumor cells (DTC) at diagnosis, ypTN and tumor regression grade (TRG) following neoadjuvant treatment, and progression-free survival (PFS).

Results: Pretreatment tumor metabolite concentrations showed no significant associations to TNM, DTC, ypTN or TRG. In univariate regression analysis, high concentrations of glycine, creatine and myo-inositol were significantly associated with poor PFS, with metastasis as main PFS event. In multivariate analysis, high glycine concentration remained most significantly associated with poor PFS (hazard ratio = 4.4, 95% confidence interval = 1.4–14.3, $p = 0.008$).

Conclusions: High tumor glycine concentration was identified as adverse prognostic factor for PFS in LARC. In a patient population treated with curative intent but with metastatic disease as main PFS event further investigations of glycine as early predictor of metastatic progression and therapeutic target are warranted.

Locally advanced rectal cancer (LARC) encompasses primary tumors growing beyond the rectal wall to an extent that precludes primary surgical removal with sufficient margin. LARC patients are commonly treated with neoadjuvant chemoradiotherapy (CRT) followed by radical surgery. Major improvements in CRT and surgical techniques the past decades have resulted in low local recurrence rates [1]. Advancements are likely to build on these improvements by developing even more precise treatment regimens with individualized neoadjuvant treatment schedules and organ-preserving surgical approaches in selected patients, in order to improve outcomes while preserving or increasing quality of life. A remaining major challenge is that 30–40% of LARC patients experience poor disease outcome due to metastatic progression [2]. At present, the idea of shifting current treatment paradigms to more individualized approaches is controversial, with a major threat being the lack of biomarkers enabling reliable treatment stratification. In this regard, deciphering the biological tumor networks distinguishing organ-confined from metastatic disease may lead to identification of the critical biomarkers.

Individual tumors demonstrate considerable biologic heterogeneity as a result of ample variations in tumor microenvironmental features such as nutrient supply, pH and oxygenation, developing as consequences of alterations in the metabolic and proliferative status of tumor cells together with highly irregular vascular structures [3,4]. Tumors' unique metabolic phenotypes result from altered substrate flux between and within major metabolic pathways following their malignant transformation, due to increased energy production (rapid ATP generation), increased biosynthesis of macromolecules and tightened maintenance of the cellular redox balance. Since shifts in tumor metabolic profiles

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http://dx.doi.org/10.1016/j.radonc.2015.11.031
0167-8140/

are directly linked to their specific pathophysiology, such profiles may be exploited in assessment of individual tumor aggressiveness and metastatic propensity.

High-resolution magic angle spinning magnetic resonance spectroscopy (HR MAS MRS) is an established method for obtaining metabolic profiles of tumor biopsies. Using this technique, we previously identified metabolic features related to microsatellite instability in colon cancer [5], and compared metabolic profiles of human colorectal carcinoma xenografts with human rectal cancer biopsies [6]. Others have used HR MAS MRS for descriptive purposes in colon and rectal cancer tissue, however, their main focus has been to reveal differences between cancer tissue and non-malignant tissue [7–10]. The use of HR MAS MRS to identify metabolic markers of tumor aggressiveness and long-term therapeutic outcome in LARC remains largely unexplored.

We aimed to identify associations between pretreatment tumor metabolic profiles and therapeutic outcome in a cohort of 54 LARC patients with completed 5 years of follow-up after receiving combined-modality neoadjuvant treatment and radical surgery.

Materials and methods

Patients and treatment

The study protocol was approved by the Institutional Review Board, The Regional Committee for Medical and Health Research Ethics of South East Norway, and The Norwegian Medicines Agency. The study was performed in accordance with the Helsinki declaration, requiring written informed consent for participation. Tumor biopsies from 54 patients enrolled between October 2005 and May 2008 were subjected to HR MAS MRS. Study eligibility criteria are listed in Appendix A. Patient and tumor characteristics are provided in Table 1. All patients received neoadjuvant chemotherapy (NACT) followed by long-course CRT and surgery (details in Appendix B).

Table 1
Patient and tumor characteristics.

No. of patients	54
Gender	
Male	35 (64.8%)
Female	19 (35.2%)
Median age (years)[a]	61 (31–73)
Male	61 (31–73)
Female	61 (38–70)
Median tumor volume at diagnosis (cm^3)[a]	16.7 (1.1–293.4)
Disseminated tumor cells, DTC[b]	
DTC positive	25 (46.3%)
DTC negative	19 (35.2%)
Not analyzed	10 (18.5%)
TNM stage at diagnosis[c]	
T2	4 (7.4%)
T3	31 (57.4%)
T4	19 (35.2%)
N0	7 (13.0%)
N1	9 (16.6%)
N2	38 (70.4%)
M0	50 (92.6%)
M1	4 (7.4%)
TN stage after neoadjuvant therapy[d]	
ypT0	13 (24.1%)
ypT1	6 (11.1%)
ypT2	12 (22.2%)
ypT3	13 (24.1%)
ypT4	10 (18.5%)
ypN0	39 (72.2%)
ypN1	12 (22.2%)
ypN2	3 (5.6%)
Tumor regression grade, TRG[e]	
1 (good response)	12 (22.2%)
2 (good response)	30 (55.6%)
3 (poor response)	5 (9.3%)
4 (poor response)	6 (11.1%)
5 (poor response)	1 (1.9%)
Follow-up results[f]	
Local recurrence	2 (3.7%)
Metastatic disease	18 (33.3%)
Death	10 (18.5%)

Note: Except where indicated, data are numbers of patients, with percentages in parentheses.

[a] Numbers in parentheses are ranges.

[b] Disseminated tumor cells in bone marrow were assessed by immunomagnetic selection at the time of diagnosis.

[c] Assessed with magnetic resonance imaging or computed tomography according to the tumor-node-metastasis system.

[d] Determined by histopathological evaluation of the surgical specimens.

[e] Tumor regression grade (TRG) is classified into good (TRG1–2) and poor histologic response (TRG3–5).

[f] Censored at a median follow-up time of 64 months (range 3–66).

Pretreatment tumor biopsies

At the time of diagnosis, 3–5 tumor biopsies were collected by rigid proctoscopy, snap-frozen in liquid nitrogen and stored at −80 °C. In order to assess tumor content in each biopsy, all biopsies were embedded in tissue freeze medium (OTC) and one 4-µm section was cut from the samples' central part using a cryostat microtome. The percentage of tumor cells in each section was assessed after staining with hematoxylin and eosin (HE). To include a biopsy in the metabolic profiling analysis we applied a cut-off of 20% as the minimum required percentage of tumor cells. For each patient, the biopsy with the highest tumor content was selected. All patients had at least one sample with tumor content >20%.

Metabolic profiling of tumor biopsies

Tumor metabolic profiles were generated by HR MAS MRS [6]. The samples were prepared on an ice block within approximately 10 min. Sample preparation involved tissue cutting to remove OTC and to fit the cylindrical MAS rotor (25 µl; mean sample weight: 13.2 mg), and adding 3 µl phosphate buffered saline in D_2O containing formate and trimethylsilyl tetradeutero-propionic acid (TSP). The HR MAS MR spectra were acquired on a Bruker Avance DRX600 spectrometer equipped with a $^1H/^{13}C$ MAS probe with gradient aligned with the magic angle axis (Bruker BioSpin GmbH, Germany). Samples were kept at 4 °C and spun at 5 kHz. Magnet shimming provided optimized line shape and minimized linewidth of formate (mean line width: 1.7 Hz).

Electronic REference To access In vivo Concentrations (ERETIC) single pulse spectra with water suppression were acquired for quantification as previously published [11]. Briefly, spectrum acquisition took 30 min for each sample. The raw spectral data were multiplied with a 0.3 Hz exponential line broadening before Fourier transformation and application of a second order linear baseline correction. Peak areas were calculated using curve fitting (PeakFit, Seasolve, USA). Metabolite tissue concentrations were calculated from the areas relative to the ERETIC signal, the ERETIC signals' previously determined concentration, and the sample weight, according to the PULCON principle [12].

Histopathologic evaluation of profiled tumor biopsies

To ensure that the profiled tumor samples contained tumor cells, all samples were subjected to histopathological evaluation. First, the tissue was preserved in 10% formalin immediately after

Please cite this article in press as: Redalen KR et al. High tumor glycine concentration is an adverse prognostic factor in locally advanced rectal cancer. Radiother Oncol (2015), http://dx.doi.org/10.1016/j.radonc.2015.11.031