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ANATOMICAL PATHOLOGY

High intratumoural but not peritumoural inflammatory host response is associated with better prognosis in primary resected oesophageal adenocarcinomas

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Summary

The host inflammatory response plays an important role in many solid malignancies. Studies on oesophageal adenocarcinomas (EACs) point towards a beneficial role of pronounced immunoreaction, however, congruent results have yet to be obtained.

We analysed 111 primary resected EAC using a tissue microarray containing three cores of the tumour centre and the periphery per case. Overall inflammation was assessed by histomorphology. Tumour infiltrating lymphocytes (TILs) were characterised by immunohistochemistry for CD3, CD8 and FoxP3, and evaluated by image analysis (Aperio ImageScope).

High levels of inflammation in the tumour centre, but not the periphery were associated with better patient survival ($p = 0.001$), similar to high counts of intratumoural FoxP3+, CD3+, CD8+ TILs ($p = 0.001$; $p = 0.027$; $p = 0.038$) and a combination of CD3+/CD8+/FoxP3+ TILs, the latter displaying three different prognostic groups (triple high/mixed/triple low; p=0.003). Intratumoural inflammation [hazard ratio (HR) = 0.432; $p = 0.030$], FoxP3+ TIL counts (HR = 0.411; $p = 0.033$) and the combination CD3+/CD8+/FoxP3+ TILs (HR = 0.173; $p = 0.006$) were also independent prognostic parameters.

In summary, both high grade total inflammation and high TIL counts in the tumour centre, but not the tumour periphery, show a beneficial prognostic impact on EAC. This may be a target for novel therapeutic options but also serves as prognostic indicator in these tumours.

Key words: Oesophageal adenocarcinoma; CD8; CD3; FoxP3; tumour infiltrating lymphocytes; inflammation.

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INTRODUCTION

The inflammatory microenvironment, which surrounds neoplastic cells, has emerged as one of the hallmarks of cancer. This has been investigated in numerous studies in recent years, confirming the important role of the inflammatory host reaction for several cancers.[1,2] In gastrointestinal cancers, most data are available for colorectal cancer, where both overall inflammation as well as the particular role of tumour infiltrating (T)-lymphocytes (TILs) and the specific subtypes of cytotoxic CD8+ T-lymphocytes and regulatory FoxP3+ lymphocytes have been analysed.[3–8] Congruent findings about the beneficial role of high TIL counts have also promoted the introduction of prognostically relevant classification schemes for colon cancer, encompassing morphology based grading systems and sophisticated classification systems using immunohistochemistry (IHC) and digital evaluation.[8–14]

Oesophageal adenocarcinomas (EACs) are highly aggressive gastrointestinal malignancies with increasing incidence, especially in Western and recently also in Eastern countries.[15] Despite improvements in surgery and the introduction of multimodal therapy concepts for advanced tumours, the prognosis of patients is still unfavourable with a 5-year survival of less than 20% when including all tumour stages.[16] For metastasised or recurrent disease, treatment options are even more limited due to a high rate of resistance to conventional chemotherapeutics, underlining the need for alternative therapeutic options.[16] On the other hand, accurate methods for risk stratification for resectable tumours would be helpful for optimising individualised therapy.

For EAC, data about the role of inflammatory host response are available to a far less degree compared to colorectal cancer. Although the results of these studies point towards a beneficial impact of intra- or peritumoural inflammatory reaction, its independent prognostic significance is still not clear. Data especially regarding the role of the different subtypes of TILs are still incongruent, which may be due in part to different methods for the characterisation of the inflammatory host reaction and differences between the patient cohorts and case collections.[17–21]

We investigated the inflammatory host reaction and its prognostic impact in a homogenous Western patient collection of primary resected EAC. The impact of the immunoreaction in the tumour centre (intratumoural) and the tumour periphery (including the adjacent peritumoural area) was analysed by both basic histomorphology with routine haematoxylin and eosin (H&E) staining and IHC, characterising different subtypes of T-lymphocytes, in correlation with clinicopathological features.

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METHODS

Patients

Formalin fixed, paraffin embedded (FFPE) tumour samples from 111 consecutive patients with primary resected EACs were included. The patients were treated in the Department of Visceral Surgery and Medicine, Inselspital Bern, with primary resection without neoadjuvant chemotherapy or radiochemotherapy between 1993 and 2011. The use of archival FFPE tissue for tissue microarray (TMA) analysis was approved by the local ethics commission (Kantonale Ethikkommission Bern, Switzerland).

Mean age of the patients was 65 years (range 31–89). Female:male ratio was 15:96. Thirty-three tumours were pT1, 10 tumours pT2, 65 tumours pT3 and three tumours pT4 according to the UICC 7th edition.[22] Lymph node metastases were absent in 52 patients and present in 59 patients. Metastases at the time of surgery were recorded in four patients. Tumour differentiation was G1 or G2 in 64 cases and G3–G4 in 47 cases. For extended information about the pathological features of the case collection, see Table 1.

Construction of ngTMA

A next generation tissue microarray (ngTMA) was constructed as described before.[23–26] In brief, annotations for the TMA cores were made on a scanned H&E stained slide that contained the tumour centre and the periphery using a Pannoramic P250 scanner (3DHistech, Hungary). Three 0.6 mm cores (total area 0.849 mm^2) were randomly selected each from the tumour centre and the tumour periphery with adjacent peritumoural areas. The TMA was then constructed using an automated tissue microarrayer (Grandmaster; 3DHistech).

Morphological assessment of inflammatory host response

The inflammatory host response was determined morphologically using routine H&E staining of the ngTMA according to the proposal of Brown *et al.*:[27] overall inflammation, including neutrophils, lymphocytes and plasma cells, was categorised as sparse, moderate or pronounced (Fig. 1A–C). Two independent reviewers performed the analysis and diverging results were discussed on a multi-head microscope to reach consensus. The three cores of the tumour centre and the periphery were first evaluated separately. Then, an overall inflammation score was built from these two categories.

Table 1 Clinicopathological characteristics of the case cohort

Parameter	Total	%
Age	Mean 65 (range 31–89)	
Gender		
Female	15	13.5%
Male	96	86.4%
pT category		
pT1	33	29.7%
pT2	10	9.0%
pT3	65	58.6%
pT4	3	2.7%
Lymph node metastases		
Absent	52	46.8%
Present	59	53.2%
Distant metastases		
Absent	107	96.4%
Present	4	3.6%
Lymphatic vessel invasion		
Absent	33	29.7%
Present	78	70.3%
Venous invasion		
Absent	82	73.9%
Present	29	26.1%
Perineural invasion		
Absent	66	59.6%
Present	45	40.4%
Grading		
G1–G2	64	57.6%
G3–G4	47	42.4%
Lauren classification		
Intestinal	73	65.8%
Non-intestinal	38	34.2%
Resection status		
R0	104	96.4%
R1	7	3.6%

IHC evaluation of T-cell infiltrates

IHC for the characterisation and quantification of T-cell infiltrates was performed on an automated immunostainer Bond III (Leica Biosystems, Germany) using the following antibodies and conditions: anti-CD3 (clone SP7, tris buffer, 95°C 30 min, 1:400; Abcam, UK), anti-CD8 (clone C8/144B, tris buffer, 95°C 20 min, 1:100; Dako, Denmark), anti-FOXP3 (clone 236A/E7, citrate buffer, 30', 100°C 30 min, 1:100; Abcam) as described before.[23,25] After staining, the slides were scanned and analysed using the Aperio ImageScope 12.2 software (Leica Biosystems) (Fig. 1D–F). The total number of positive cells across the TMA cores was recorded. The sum of the cell counts of the three cores of the tumour centre and the periphery was then used as TIL count for the respective tumour areas. The optimal algorithms for each immunostaining had been established in a preceding study by the comparison of the quantification results between manual counting of the stained cells in the TMA cores and the Aperio software, with a high intraclass correlation (ICC > 0.79) between the two quantification methods. The algorithms are provided in Supplementary Tables 1–3 (Appendix A).[28]

IHC analysis for mismatch repair deficiency

IHC for mismatch repair (MMR) proteins was performed on the Leica Bond III autostainer as follows: MLH1 (clone ES05, tris buffer 95° 30 min, 1:200; Novocastra, UK); MSH2 (clone G219-1129, tris buffer 95° 30 min, 1:500; CellMarque, USA); PMS2 (clone A16-4, tris buffer 95° 30 min, 1:100; BD Bioscience, USA), and MSH6 (clone PU29, tris buffer 95° 30 min, 1:50; Novocastra).

Tumours were classified into MMR-proficient cases expressing MLH1, MSH2, PMS2 and MSH6, or MMR-deficient cancers with absence of the expression of one of more of these proteins.[29]

Statistical analysis

The software SPSS v23 (SPSS, USA) was used for statistical analysis. Correlation analysis was performed using Spearman's Rho test. For comparison of groups, chi-square and Fisher tests were used. Differences between variables with continuous scores were calculated using non-parametric tests (Mann–Whitney U test). Survival data were available from 91 patients. For survival analysis Kaplan–Meier curves and log-rank tests were used for univariate analysis. Multivariate analysis was performed using Cox regression analysis. The significance level was set for a p value <0.05.

RESULTS

Overall inflammation

Data regarding intratumoural and peritumoural inflammation were available for 111 and 98 cases, respectively. The reduced number of cases was due to small tumour size or technical reasons (loss of TMA cores; lack of tumour or insufficient tumour tissue in TMA cores). Intratumoural and peripheral inflammation showed a significant positive correlation ($p < 0.001$). Inflammation was sparse in 55 cases, moderate in 53 cases and pronounced in three cases. Peritumoural inflammation was categorised as sparse in 39 cases, moderate in 51 cases and pronounced in eight cases. For further analysis, moderate and pronounced inflammation was merged into high grade, in contrast to sparse that was assigned as low grade inflammation.

Tumour infiltrating lymphocytes

Data for intratumoural lymphocytic counts were available for all 111 cases and for peritumoural lymphocytes for 108 cases. The distribution of all TIL groups (CD3+, CD8+, FoxP3+) showed a wide range (CD3+TIL intratumoural 1-231/