Abstract. Adenosquamous carcinoma (AdSqCa) is a rare aggressive subtype of colorectal carcinoma with interesting issues concerning histogenesis, prognosis and appropriate management. We analyzed 9 cases of colonic AdSqCa diagnosed in our department in the last 4 years from a total of 330 cases of colonic epithelial malignancies. Histological features were reviewed and histochemical and immunohistochemical studies were performed in order to highlight specific characteristics of the tumors. The gross appearance of carcinomas varied within large limits but all of them showed the same rectosigmoidian location. Most of the cases had high grade of anaplasia as well as other histopathological signs of tumoral aggressiveness: ulceration, necrosis, perivascular and perineural invasion, tumoral emboli, extension to the entire colonic wall. Moreover, 3 patients presented advanced stage disease – either regional lymph node or liver metastasis. The cytonuclear pleomorphism was more pronounced and the proliferation indexes were higher in the squamous component than in the pure adenocarcinomatous counterpart. Its high biological aggressiveness should draw attention to the importance of correct diagnosis of these rare colonic neoplasms. We report our experience regarding an increased incidence and exclusive rectosigmoidian location of AdSqCa, singular features of this type of tumor identified in medical literature. Moreover, the overall histopathological appearance and the immunohistochemical phenotype of the analyzed cases sustain the hypothesis of squamous metaplasia of the glandular adenomatous epithelium with subsequent malignant transformation as possible mechanism of AdSqCa histogenesis.

Keywords: adenosquamous carcinoma, colorectal carcinoma

Introduction

Adenosquamous carcinoma (AdSqCa) of the colon and rectum is an extremely rare tumor with an overall incidence of 0.025-0.1% among colonic neoplasms. As a direct consequence of this low incidence, the data referring to the pathogenesis, clinical and pathological evolution, prognosis and treatment are incomplete [2,6,14].

Material and method

We analyzed the patients with colonic carcinoma diagnosed on endoscopic and/or surgical material in the Department of Pathology, Colentina University Hospital. The surgical specimens were macroscopically examined and several tissue fragments were selected for further microscopic assessment, according to the recommended protocols [13]. After prelevation, tissue fragments were fixed in buffered formalin 10% (formaldehyde 3.7%) for 24-72h and manually histopathologically processed (dehydration by immersion in ethanol solutions with increasing concentrations of 90 for 24h, 96 for the next 24h, 100 for 24h, clarification in toluene for 24h and paraffin impregnation 24h at 56°C). The paraffin
impregnated fragments were embedded in paraffin blocks.

Paraffin–embedded tissue blocks were cut with a semi-automatic rotative microtome (3 μm-thick sections for routine and histochemical stains - hematoxylin-eosin HE, Van Gieson VG, periodic acid Schiff PAS). After histopathological examination, 9 cases of AdSqCa were selected for further immunohistochemical (IHC) tests. 2 μm-thick sections were displayed on poly-L-lysine pretreated slides and dried for 2h in a 56°C oven. When IHC stains were performed later than 3 days since the sections were cut from the paraffin block, the blanks were covered by paraffin in order to prevent loss of antigenicity due to oxidation. Several IHC markers were performed: pancytokeratin AE1-AE3, CK7, CK20, CK8-18, CA19-9, 34βE12, Ber-EP4, cytokeratin with high molecular weight DESQ, p63, ki67; all the IHC markers were provided by Labvision as ready to use products; a tristrial method was used, the antispecies secondary antibodies, streptavidin peroxidase and chromogen (DAB) being also provided by Labvision.

Results

We report 9 cases of AdSqCa of the colon diagnosed in our department between 2005-2008 from a total of 330 cases of colonic carcinomas (2.72%). The main clinical and histopathological data are summarized in Table I. There was a female predominance (7 cases – 77.77%) with a median age of 63 yrs (the extremes of age varied between 32 and 82 yrs). All the cases were located in the rectosigmoid area (4 cases within the rectum – 44.44%, 4 cases within the sigmoid – 44.44% and one case in the rectosigmoid junction 11.11%). All but one tumor were treated by open surgery, the lesion in the remaining case being endoscopically resected. The surgically resected specimens showed big tumors (4 up to 11 cm in the biggest diameter, median diameter 7.6 cm); the endoscopically resected lesion had smaller dimensions (1.4/1.2/1.2 cm).

RS junction: rectosigmoid junction
0 – absent
1 – mild
2 – moderate
3 – marked

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Histopathological examination revealed biphasic malignant cellular proliferation with glandular and squamous components, either intricate or separate (Figure 1). Two cases consisted of almost exclusive squamous proliferation with minute areas of mucin production in isolated tumor cells better identified by histochemical stains (alcian blue) (Figure 2). Half of the cases presented mucin lake formation. Occasional keratin whorls were identified in some cases.

Figure 1. Separate areas of adenocarcinomatous and squamous components of AdSqCa. HE, original magnification x 100

Most of the cases had high grade of anaplasia (G3 7 cases – 77.77%), the cytonuclear pleomorphism was more pronounced in the squamous component than in the adenocarcinomatous one (Figure 3). One patient was initially treated for a colonic adenocarcinoma (thorough retrospective examination of several tumor fragments failed to reveal any area of squamous differentiation) and later on, after a 2yrs interval free of disease, a recurrence occured with histopathological appearance of pure AdSqCa. One other patient had synchronous tumors; he presented a gastric tumor resected 5 months after the colonic resection.

Figure 2. Tumoral areas with almost exclusive squamous phenotype – large polygonal cells with distinct cellular borders, abundant eosinophilic cytoplasm and vesicular nuclei. HE, original magnification x 40.

Figure 3. Squamous component with marked cytonuclear pleomorphism and numerous atypical mitoses. HE, original magnification x 200.

There was no dysplasia of the nontumoral colonic mucosa in the vicinity of the tumor (brisk transition from the normal glandular epithelium towards the squamous tumor proliferation). One patient presented small tubulovillous adenoma in the vicinity of the tumor: the adenoma presented low grade intraepithelial neoplasia with small areas of squamous metaplasia of the dysplastic epithelium. None of our patients presented ulcerative colitis or other inflammatory bowel diseases.

All these tumors presented histopathological signs of aggressive tumors – ulceration (8 cases – 88.88%), moderate-marked necrosis (moderate necrosis 2 cases – 22.22%, marked necrosis 6 cases – 66.66%), perivascular invasion (all the cases), perineural invasion (6 cases – 66.66%), tumor emboli (6 cases – 66.66%). All patients with

Figure 4. The squamous component was strongly DE-SQ immunoreactive, while the normal and tumoral glandular areas were negative. DE-SQ, original magnification x 40.
surgical resection presented highly invasive tumors (stage pT3), 3 patients presenting advanced stages of disease (2 patients with numerous lymph node metastasis pN2, one other patient with distant metastasis pM1). Most of the cases had moderate/marked intratumoral inflammatory infiltrate (88.88%), all the cases moderate/marked tumor desmoplasia.

All the tumor cells were positive for AE1-AE3 and CK7. CK8-18, CK20 and CA19-9 were intensely positive in adenocarcinomatous areas; CK 20 was negative in squamous/adenosquamous parts of the tumors while CK8-18 and CA19-9 were faintly positive in these areas. DESQ, 34βE12, Ber-EP4 and p63 were negative in adenocarcinomatous areas and positive in squamous/adenosquamous areas (markers for squamous differentiation) but the positivity was focal (Figure 4); however, in adenosquamous areas these IHC markers were positive both in cells with squamous morphology and in cells with prominently undetermined morphologic phenotype. Ki67 (marker of proliferation) had higher indexes in squamous/adenosquamous areas than in pure adenocarcinomatous areas.

**Discussion**

We analyzed the types of colonic carcinomas diagnosed in our department during the last 10 yrs. The 9 cases of AdSqCa we report here were diagnosed in the last 4 yrs of the selected period, no other cases being diagnosed in our department since 1999. The overall incidence of AdSqCa was 2.72% since 2005. We were surprised to discover such a high incidence of this type of tumor in the latest years and we question this incidence as being artificially increased due to unidentified artifacts [15,16].

First, we considered a possible interobserver error based on various interpretation of the proportion of the squamous/adenosquamous proliferation within the total tumor mass. There is no precise specification in field literature on the minimal quantity of squamous differentiation within a colonic adenocarcinoma in order to call that lesion AdSqCa. It is possible that one observer will call a lesion AdSqCa while another will label the same lesion adenocarcinoma with a note of minute areas of squamous metaplasia accompanying the tumor.

In our series, all the AdSqCa cases showed squamous differentiation in large areas of the tumor (in fact in two cases special histochemical stains and IHC markers were performed in order to confirm the adenocarcinomatous component of the tumor). Thus, we were convinced that we had not overdiagnosed ordinary adenocarcinomas with minute areas of squamous component as AdSqCa. Then we searched earlier reports for squamous differentiation of the tumor cells: no such mentions were identified, the hypothesis of overlooking a diagnosis of AdSqCa being thus excluded.

Then we considered a possible sampling error. During macroscopic sampling of all the AdSqCa, 3 to 5 fragments of tumor were selected for each case (except for the endoscopically resected cases that was completely embedded in 2 blocks); the tumor fragments were generous (2/2.5 to 2.4/3 cm), allowing the examination of a large area of tumor. There was no difference regarding the method of sampling before 2005, at least 3 fragments of tumor being selected for microscopic examination in every tumor; in fact, several cases were extensively sampled (more than 6 tumor fragments per case). One can argue that areas of squamous differentiation in an otherwise ordinary adenocarcinoma can be missed if the tumor tissue is not embedded in toto (i.e. AdSqCa cannot be completely ruled out without microscopic examination of the whole tumor); however, since we had not recently changed our method of macroscopic investigation, there is no reason to think that random sampling favored the identification of AdSqCa. The possibility of overlooking areas of squamous/adenosquamous differentiation was thus reasonably excluded.

The origin of the AdSqCa is still on debate, several hypotheses being considered: 1. squamous metaplasia of the glandular epithelium of colorectal adenomas with subsequent malignant transformation or squamous metaplasia of the adenocarcinomatous cells; 2. neoplastic transformation of uncommitted pluripotent stem cells with biphasic differentiation, both epithelial and squamous; 3. neoplastic transformation of ectopic embryonic cell with ectodermic origin. The overall histopathological appearance of the tumors we evaluated seems to favor the first theory. Five of our cases presented large areas of pure adenocarcinomatous proliferation with areas of transition towards squamous/adenosquamous areas and one case with pure adenosquamous proliferation had in close vicinity a small tubulovillous adenoma with areas of squamous metaplasia (similar findings were reported by other authors [7]).

Our report discusses the histopathological
characteristics of the AdSqCa. All the cases were aggressive tumors. One case was identified as a polypoid mass that was endoscopically resected; the level of invasion was quite deep for a polypectomy—submucosal invasion, stage pT1. All the other cases were surgically resected, the tumors extending beyond muscularis propria into the pericolonic fat (stage pT3); 3 cases also presented local or distant spread of disease (2 cases pN2, one case pM1). All the cases showed microscopic stigmata of aggressive tumor: perivascular and/or perineural invasion, tumor emboli, ulceration and tumor necrosis. Interestingly, almost all cases showed intratumor inflammatory infiltrate and desmoplasia, features that are usually encountered in tumors with early clinical symptoms. The co-occurrence of these early clinical heralds and advanced stage of disease at presentation underline the biological aggressiveness of this type of tumor [1,3,4].

None of our cases had cecal localization as suggested by other authors [17]. All the patients presented with left-side colonic tumors, in fact all of the cases were confined within the sigmoid and rectum. There are cases reported with similar location [4,11,12] but, at least in our knowledge, this is the first series with so numerous cases of AdSqCa with exclusively rectosigmoid location. We do not have an explanation for these findings. We excluded the possible extension of a squamous tumor from the anal area and we also excluded metastases, lesions suggested by other authors as possible causes of missdiagnosis [5,8,9]. Several authors reported an association between AdSqCa and ulcerative colitis [10]. None of our cases presented inflammatory bowel disease associated to the neoplastic lesions.

In conclusion we present a series of 9 cases of AdSqCa of the sigmoid and rectum with unusually high incidence. We were not able to find explanations for the frequency of the lesion or for the unusual localization but we confirm the higher biological aggressiveness of this type of colonic carcinoma.

References