BRAF Mutation Is Reduced In Anal Compared To Cutaneous Melanoma

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BRAF Mutation Is Reduced In Anal Compared To Cutaneous Melanoma

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Abstract

Study objective: Melanoma of the anal mucosa is a rare and aggressive cancer. There is little evidence about the optimal investigation and treatment of this condition. The aims of this study were to investigate the prevalence of BRAF mutation in anal melanoma (AM), compared to that of the cutaneous form of the disease (BRAF mutation present in around 60% of cases). Secondary objectives were to assess overall survival and recurrence rates.

Methods and procedures: An observational study with retrospective data collection was conducted on all patients coded with AM on the local pathology system. Data were collected on patient demographics, radiological investigations, local and systemic therapies, pathological reports and mortality and morbidity. BRAF mutation was assessed through a newly validated immunohistochemistry (IHC) assay.

Results of study: Nine patients were diagnosed with AM over the study period, 7 had tissue available for BRAF IHC. There were five men, and two women, with a median age of 66 years (range 48–74 years). The mortality rate was 71.4% (5 of 7 patients), with a one and two-year survival of 57% and 29% respectively. Of the seven patients tested for a BRAF mutation, one (14%) tested positive for a mutation in the BRAF gene on IHC.

Conclusion: AM is an extremely aggressive cancer with very poor prognosis. This study demonstrated a low incidence of BRAF mutation in AM compared to cutaneous melanoma, with very poor survival.

KEYWORDS: melanoma, anal, BRAF, mutation, gene, cancer
Introduction

Melanoma of the anus is a rare, aggressive tumour originating from the embryologic neural crest melanocyte cells. It accounts for around 0.5-2% of all anorectal malignancies. The anus is the commonest site for primary gastrointestinal melanoma [1] and the third commonest site for all melanoma, behind cutaneous and ocular forms of the disease [2-4]. Risk factors for the cutaneous form of the disease include exposures to ultraviolet B radiation (UVB), a fair skin complexion, and xeroderma pigmentosum, however these have no known association with anal melanoma (AM) [5]. Due to the rarity of the disease, data on the epidemiology of the disease is generally lacking. Table 1 summarises the published series on AM. Reported cases of rectal or anorectal melanoma were discounted due to a different disease aetiology [3, 6-9].

The overall 5-year survival for AM patients is less than 10%, and survival in patients with systemic or recurrent disease is less than 10 months [10, 11]. There are no reported cases of long-term survival in patients with advanced disease.

Table 1. Previous reports on anal melanoma

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>41</td>
<td>109</td>
<td>12</td>
<td>255</td>
<td>126</td>
</tr>
<tr>
<td>Median Age (yrs)</td>
<td>61</td>
<td>70</td>
<td>67</td>
<td>58</td>
<td>69</td>
</tr>
<tr>
<td>Male Sex (%)</td>
<td>39%</td>
<td>35%</td>
<td>25%</td>
<td>47%</td>
<td>31%</td>
</tr>
<tr>
<td>Stage III (systemic disease)</td>
<td>34%</td>
<td>42%</td>
<td>8%</td>
<td>38%</td>
<td>17%</td>
</tr>
<tr>
<td>BRAF mutation</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>OS, months (median, IQR)</td>
<td>22 (18-36)</td>
<td>23 (20-25)</td>
<td>nr</td>
<td>nr</td>
<td>15 (nr)</td>
</tr>
</tbody>
</table>

*nr= not reported, OS=overall survival, IQR=interquartile range

Recent studies in cutaneous melanomas have demonstrated that mutations in the gene encoding the serine-threonine protein kinase B-RAF (BRAF) occur in around 60% of cases [14]. Mutations of this gene activate the mitogen-activated protein kinase (MAPK) pathway, which prevents cellular apoptosis and promotes cell proliferation [15]. Following this work, vemurafenib and dabrafenib were developed as potent kinase...
inhibitors with specificity for the BRAF V600E mutation within cancer cells, which will benefit around half of these patients. There is evidence of an increased 6-month survival (84% vs. 64%) compared to standard treatment [16, 17]. The aims of this study were to investigate the prevalence of BRAF mutation in anal melanoma (AM), compared to that of the cutaneous form of the disease (BRAF mutation present in around 60% of cases). Secondary objectives were to assess overall survival and recurrence rates.

**Methods**

All patients with AM treated in our unit between 1999 and 2014 were identified from the local pathology system. Our centre is a tertiary referral centre for both anal cancer and melanoma. All patients, including those undergoing palliative resection were included. Patients were considered to have primary AM after radiological and histopathological staging, when no other primary site could be found and the tumour arose within the anal canal. Melanomas of the rectum, anal margin or perineum were excluded. The inclusion of occult melanoma with anal metastases was minimised by using CT scans of head, chest, abdomen and pelvis pre-operatively.

BRAF V600E mutation was assessed with immunohistochemistry (IHC) using the mutation specific BRAF V600 VE1 antibody (Ventana, Roche, Burgess Hill, UK). This test was done on all patients with AM where tissue was available. The methodology has previously been described [18]. The technique was independently verified in our institution by performing IHC along side molecular testing on 13 cutaneous melanoma samples.

Apart from BRAF status, overall survival (OS), disease free survival (DFS) and local, regional and distant recurrence as well as postoperative morbidity and mortality were recorded. Follow-up data was collected up to the point of death, or the last known data available on a patient. The staging of AM is different from the cutaneous form, in that the tumour thickness is not considered. Table 2 outlines the characteristics of each tumour stage (I-III)[19]. The staging was determined by a combination of histological and radiological examination. Surgery was defined as R0 when the margins were microscopically negative, R1 when it was microscopically involved and R2 when macroscopically involved. Categorical variables were compared using $\chi^2$ test. Statistical significance was assumed at the 5% level.

**Table 2. Anal melanoma staging**
<table>
<thead>
<tr>
<th>Tumour Stage</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Localized disease</td>
</tr>
<tr>
<td>II</td>
<td>Inguinal or pelvic lymph node disease</td>
</tr>
<tr>
<td>III</td>
<td>Distant metastases</td>
</tr>
</tbody>
</table>

Results

Nine patients were diagnosed with AM over the study period, but only 7 (five men and two women) had tissue available for BRAF IHC. Tissue was unavailable for two patients as they had their surgery more than five years ago, and no tissue was stored passed this point. The median age was 66 years (range 48–74 years). At the time of surgery, two patients had stage I disease, two had stage II disease and two already had systemic disease (stage III). One patient had no pre-operative imaging of the pelvis, therefore the differentiation between stage I and II disease could not be made. Five patients had a wide local excision and two had an Abdomino-Peroneal Excision of Rectum (APER). One patient that presented with stage III disease and had a WLE had macroscopically positive margins (R2). All other patients had microscopically clear margins (R0). Six of seven patients were a diagnostic surprise (85.7%), where the diagnosis was not suspected until the pathology report was available. All patients having radical surgery (APER) had the diagnosis confirmed by biopsy before the decision to operate was made.

BRAF IHC was performed on all patients with tissue available, one patient, a 48 year-old male tested positive for a BRAF mutation (14.3%). A summary of each patient is given in Table 3. Five pathology reports stated the depth of tumour invasion, with a median of 9mm (range 2.9-36mm). There was no relation between tumour thickness and stage of presentation (p=0.423). Only two of the seven patients are still alive at the time of data analysis (309 and 5095 days post diagnosis). All deaths were melanoma related. The mortality rate was 71.4% (5 of 7 patients), with a one and two-year survival of 57% and 29% respectively. The median survival was only 404 days post-op (range 69-5096 days). There were no intraoperative deaths, and as expected the mean hospital stay was much shorter for WLE (0 days) compared to APER (14.5 days). There were no major surgery related complications in the two patients having APER in terms of morbidity or mortality.
**Table 3. Anal melanoma patient and tumour characteristics**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Stage</th>
<th>Operation</th>
<th>Length Of Stay (days)</th>
<th>Resection</th>
<th>Tumour Thickness (mm)</th>
<th>Neoadjuvant Radiotherapy</th>
<th>Metastases</th>
<th>Location of Metastases</th>
<th>Mortality</th>
<th>DFS (days)</th>
<th>Survival (days)</th>
<th>BRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>III</td>
<td>WLE</td>
<td>0</td>
<td>R2</td>
<td>2.9</td>
<td>0</td>
<td>1^</td>
<td>Lung</td>
<td>Dead</td>
<td>0</td>
<td>404</td>
<td>Mut</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>III</td>
<td>WLE</td>
<td>0</td>
<td>n/a</td>
<td>2.7</td>
<td>1</td>
<td>1^</td>
<td>Liver</td>
<td>Dead</td>
<td>0</td>
<td>69</td>
<td>Wt</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>II</td>
<td>WLE</td>
<td>0</td>
<td>R0</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>Lung, Bone</td>
<td>Dead</td>
<td>526</td>
<td>724</td>
<td>Wt</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>I</td>
<td>WLE</td>
<td>0</td>
<td>R0</td>
<td>4.7</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>Alive</td>
<td>5095</td>
<td>5095</td>
<td>Wt</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>II/III</td>
<td>WLE</td>
<td>1</td>
<td>R0</td>
<td>nr</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>Alive</td>
<td>309</td>
<td>309</td>
<td>Wt</td>
</tr>
<tr>
<td>6</td>
<td>74</td>
<td>I</td>
<td>APER</td>
<td>13</td>
<td>R0</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>Brain</td>
<td>Dead</td>
<td>99</td>
<td>142</td>
<td>Wt</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>II</td>
<td>APER</td>
<td>16</td>
<td>R0</td>
<td>36</td>
<td>0</td>
<td>1</td>
<td>Lymph Nodes</td>
<td>Dead</td>
<td>144</td>
<td>1236</td>
<td>Wt</td>
</tr>
</tbody>
</table>

n/a= not applicable, nr= not reported, DFS= disease free survival, WLE= wide local excision, APER= abdomino-peroneal excision of rectum, Wt= BRAF wildtype, Mut= BRAF mutation, ^= metastases on presentation
Discussion

In the medical literature, the rates of BRAF mutation seen in cutaneous melanoma are between 49-74% [16, 20, 21]. In this study utilizing immunohistochemical techniques, only one of seven (14.3%) patients had this mutation, which may be explained by different disease biology compared to the cutaneous form of the disease. A possible explanation for this phenomenon is that ultraviolet-B (UVB) is responsible for more than 80% of dependent non-silent coding mutations in cutaneous melanoma. These deactivating mutations occur in genes coding for GPTase (such as RAC1 and RAS oncogenes) as well as BRAF and NRAF, which in turn drive the tumour initiation and progression [22-24]. It is probable that the pathway that leads to AM is different, due to the lack of exposure to UVB. Immunohistochemical testing has a reported sensitivity of 97% (37/38) and a specificity of 98% (58/59) for detecting the presence of a BRAF V600E mutation [25].

This retrospective study has confirmed the poor prognosis after surgery for AM, no matter whether radical or local resection is undertaken. Only one patient had a five-year disease free survival, and she had Stage I disease and complete (R0) excision following a polypectomy for an anal polyp. The evidence surrounding the optimal surgical treatment for AM is limited to case series and retrospective reviews due to the rare nature of the disease, and the impracticalities of performing randomised trials of treatment. Previous authors have advocated a radical approach to AM with APER and inguinal and mesorectal lymphadenectomy [26-28], and even pelvic exenteration [29]. Others have advocated a less aggressive approach with WLE, as this is associated with less morbidity and similar outcomes [4, 9, 30]. There is evidence that if the disease is diagnosed early (Stage I) and the patients are treated with preoperative chemotherapy or radiotherapy, that better OS and DFS can be achieved with both WLE and APER [8]. As there is no level 1 or 2 evidence for the use of neoadjuvant or adjuvant therapy in AM, the standard of care in the absence of systemic disease is close observation [6]. In the presence of systemic disease, adjuvant therapy may be given.

The main weakness of this study is the small study population; this is due to the rarity of the disease. This new case series adds to the limited amount of evidence surrounding treatment for this aggressive cancer and demonstrates a lower rate (14.7%) of BRAF mutation in AM compared to the cutaneous form (60%). This suggests that potent kinase inhibitors will have little impact upon the depressing prognosis associated with AM. However, BRAF testing should still be undertaken as there may be benefits to a small amount of patients with this very aggressive cancer.
References


