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# Multidrug Resistance Proteins in Primary and Metastatic Soft-tissue Sarcomas: Down-regulation of P-glycoprotein During Metastatic Progression

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**Abstract.** *Background: Chemotherapy sensitivity of soft-tissue sarcomas (STS) is limited, which may be due to multidrug resistance (MDR). MDR is associated with expression of P-glycoprotein (P-gp), Multidrug Resistance-associated Protein 1 (MRP1) and Lung Resistance-related Protein (LRP). It is unknown whether in STS metastasis is more resistant than the primary counterpart. Materials and Methods: In 35 chemonaive STS and their metastases (86% chemonaive), MDR proteins were immunohistochemically assessed. Eleven metastases presented synchronously, 24 metachronously. Expression was scored positive (>5% positive tumour cells) or negative. Results: P-gp was positive in 31/34 primaries (91%), versus 22/32 metastases (69%) (p=0.005). This difference was significant for metachronous metastases (p=0.008). MRP1 was positive in 18/32 primaries (56%) and 22/33 metastases (67%). MRP1 was more expressed in synchronous metastases than primaries (p=0.047), but for the overall group this significance disappeared. LRP expression did not differ: 27/34 primaries (80%), versus 28/34 metastases (82%). Conclusion: P-gp, MRP1, LRP expression in the primary tumours was high. Metastatic progression did not coincide with MDR-protein up-regulation.*

Soft-tissue sarcomas (STS) are a heterogeneous group of mesenchymal tumours. Many histological types have been described, which often show differences in biological propensities and clinical behaviour. Even within a single histological type, subgroups with different biological make-up and clinical outcome can be distinguished. Taken together, almost half of the STS patients develop metastases in due course. Most of the metastases present as pulmonary lesions; lymph node metastases have been described in about 3% of STS (1). The highest incidence of lymph node metastases has been described for angiosarcomas, embryonal rhabdomyosarcomas and epitheliod sarcomas (1,2). Bone metastases are found in 7%-10 % of STS (3), predominantly in rhabdomyosarcomas. Once metastasised, cure is difficult to achieve. In cases where a limited number of metastases are present, metastasectomy can be applied with curative intent. However, for the majority of patients with metastasised STS, chemotherapy will be the only option. The relative resistance to chemotherapy of STS in adulthood is at least partly due to multidrug resistance. This nowadays well-known phenomenon covers resistance to a variety of cytotoxic drugs, such as anthracyclines, vinca-alkaloids and epipodophyllotoxins. Expression of P-glycoprotein (P-gp), the multidrug resistance protein 1 (MRP1) and lung resistance protein (LRP) play a role in this multidrug resistance. In adult STS, doxorubicin and ifosfamide are the most effective drugs.

There are a few reports studying the expression of these MDR proteins in primary tumours and metastases, which might give an indication about the correlation of P-gp, MRP1 and LRP and metastatic potential (4-7).

Because of the clinical problem of drug resistance in STS and the high expression of the multidrug resistance proteins P-gp, MRP1 and LRP in STS (8), we wondered whether there might even be an up-regulation of one or more of these proteins during the process of metastatic progression. We therefore studied paired samples of primary and metastatic STS from 35 patients treated at our hospital.

## Materials and Methods

Patients with a STS, of which samples of both the primary tumour and metastasis were available, were retrieved from the computerised files of the Department of Pathology at the University Hospital Groningen (the Netherlands).

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Sarcomas were reviewed on hematoxylin and eosin-stained paraffin-embedded sections with additional immunostains and were classified according to Enzinger and Weiss (9). Metachronous metastases were defined as metastases detected after 3 months of diagnosis of the primary tumour. Synchronous metastases were those detected within 3 months of diagnosis.

*Immunohistochemistry.* Immunohistochemistry with the indirect peroxidase method was performed as described previously (10). The most viable parts of the tumour were selected from the available blocks. The following monoclonal antibodies were used: C494 to P-gp (Signet Laboratories Inc., Dedham MA, USA; dilution 1:200); *MRPr1* to MRP1 (kindly provided by Dr R.J. Scheper, Free University Hospital, Amsterdam, the Netherlands; dilution 1:15); and LRP *clone 42* to LRP (Transduction Laboratories, Los Angeles CA, USA; dilution 1:400). As positive controls for P-gp, MRP1 and LRP, liver, lung and colon were used, respectively. Immunohistochemistry was scored by two independent observers, having no knowledge of the clinical data. The scoring was performed semi-quantitatively as positive (>5% immunoreactive tumour cells) or negative.

*Statistics.* Changes in the expression of MDR proteins were assessed by analysing the paired samples of primary tumours and their corresponding metastases by a Wilcoxon signed rank test. For statistical analysis, Statistical Package for the Social Sciences (SPSS) 10.0 for Windows (SPSS Incorporated, Chicago IL, USA) was used.

## Results

Between 1981 and 1997 paired samples of 35 STS could be retrieved. In 11 cases metastases had occurred synchronously and in 24 cases metachronously. The following histological types of STS were diagnosed: 3 liposarcomas, 3 angiosarcomas, 3 epithelioid sarcomas, 4 synovial sarcomas, 4 rhabdomyosarcomas, 4 leiomyosarcomas, 5 malignant fibrous histiocytomas (MFH), 5 sarcomas not otherwise specified (NOS) and 4 other sarcomas (1 malignant schwannoma, 1 malignant hemangiopericytoma, 1 fibrosarcoma, 1 clear cell sarcoma). The localisation of the metastases was lung in 12 (34%), lymph node in 11 (31%), soft tissue in 7 (20%), bone in 2 (6%), pleural in 2 (6%) and mesenterial in 1 (3%). Lymph node metastases were found in 2 cases of RMS, 1 epithelioid sarcoma, 1 clear cell sarcoma, 3 MFH, 1 LMS, and 2 NOS. Whereas all samples from primary STS were chemotherapy naïve, this was the case in 30 (86%) of the metastases; 5 (14%) patients had received chemotherapy, after which the metastasis was removed.

Expression of P-gp, MRP1 and LRP is summarised in Table I. P-gp was expressed in 31/34 primary tumours (91%) *versus* 22/32 metastases (69%). Paired analysis (primary *vs.* metastasis) revealed significantly less P-gp-positive samples in the metastases group ($p=0.005$). This difference seems to be due to the metachronous metastases group in which far less P-gp-positivity was found in the metastases than in the primaries ($p=0.008$). In the synchronous metastases group, no significant difference in P-gp expression was found between primaries and metastases.

Table I. *Semi-quantitative assessment of P-gp, MRP1 and LRP in primary and corresponding metastatic soft tissue sarcoma.*

| | | P-gp | MRP1 | LRP |
|---|---|---|---|---|
| Primary | positive | 31 (91%) | 18 (56%) | 27 (80%) |
| | negative | 3 (9%) | 14 (44%) | 7 (20%) |
| | missing | 1 | 3 | 1 |
| Metastasis | positive | 22 (69%) | 22 (67%) | 28 (82%) |
| | negative | 10 (31%) | 11 (33%) | 6 (18%) |
| | missing | 3 | 2 | 1 |

Wilcoxon signed ranks test shows that P-gp expression is significantly different: $p=0.005$. Both MRP1 and LRP expression are not significantly different between primary *versus* metastasis.

MRP1 was positive in 18/32 primary tumours (56%) and in 22/33 metastases (67%). LRP was positive in 27/34 primary tumours (80%) *versus* 28/34 metastases (82%).

MRP1 and LRP expression did not significantly differ in the total group of primary tumours *versus* their cognate metastases. However, subgroup analysis revealed that, in the group with synchronous metastases, MRP1 was significantly more often positive in metastases than in primary STS ($p=0.046$). This was not the case for the metachronous metastases group.

After subgroup analysis for the synchronous and metachronous metastases groups, still no difference between metastases and primaries was found for LRP.

Subgroup analysis of the chemotherapy-pretreated metastases could not be performed, due to the limited sample size.

It was found that expression of each of the three MDR-associated proteins did not differ for lymph node metastases *versus* the group of non-lymph node metastases. P-gp, MRP1 and LRP expression were the same for the group of lymph node metastases *versus* their paired primaries, as for non-lymph node metastases *versus* their primary counterparts.

## Discussion

Despite the substantial burden of literature about multidrug resistance, still several questions remain unanswered and results of studies are at times conflicting. Many papers deal with the physiological and tumour biological role of P-glycoprotein. The role of efflux pumps extruding xenobiotics from normal cells to protect them from harm and the

barrier role, *e.g.* in the blood brain and blood testis barrier, are well known (11, 12). In solid tumours, P-gp expression is a major obstacle to the effectiveness of a broad variety of natural cytotoxic agents. In STS, P-gp expression (or MDR1 gene expression, encoding for P-gp) has been related to the response to chemotherapy in childhood and adult STS (13-20). In addition, the role of P-gp in the course of metastatic progression has been postulated, but has not yet been clarified (21,22).

In this study, P-gp was expressed in a high percentage of tumour samples. In a previous study of our group we found a somewhat lower P-gp expression in primary STS (79% of the cases were P-gp-positive) (8). The difference between these studies might well have been caused by the selection of metastasising tumours and the greater share of histological types known for extensive P-gp expression in the present study, like synovial sarcomas, rhabdomyosarcomas and MFH. The finding of the significantly lower number of P-gp-expressing metastases than their matching primaries was rather unexpected. The significance of this result seems to be related to the metachronous metastases, *i.e.* metastases detected beyond 3 months after diagnosis of the primary tumour. Previously, Mattern did not find a difference in P-gp expression in lung tumours in paired primary and simultaneously resected lymph node metastases (4). In a comparable sample size as the present study, P-gp was not up-regulated in post-chemotherapy resected metastases from melanomas, when compared with the matched chemonaive primary melanomas (6). In epithelial ovarian cancer also MDR1 expression did not differ significantly between primary and metastatic sites (23). In a murine osteosarcoma tumour model MDR1 gene expression was studied during tumour progression. No significantly different levels of MDR1 gene expression were found between primary, recurrent and metastatic lesions (24). Finally, Zochbauer-Muller also observed a slightly lower expression of P-gp in lymph nodes of breast cancer patients *versus* the paired primaries (7).

MRP1 function is being increasingly explored (25). Apart from resistance to the classical MDR drugs, MRP1 can also cause resistance to methotrexate. The clinical significance of high expression of MRP1 in solid human tumours remains to be elucidated. Limited data are available on the role of MRP1 in STS. Oda *et al.* found a relationship between the expression of MRP1- RNA and the malignancy grade of STS (26). Previously, MRP1 expression was found in 67/136 primary STS (49%) (8), which is an only slightly lower percentage than the 56% MRP1-positive samples presented here. The former study consisted of a relatively higher share of leiomyosarcomas and myxoid liposarcomas, which types turned out to be MRP1-negative in a substantial number of cases (8).

Although a subgroup analysis showed an increase in MRP1 for synchronous metastases (which was not observed for metachronous metastases), this was of borderline statistical significance. Of interest, Zochbauer-Muller observed, in breast cancer patients, elevated MRP1 expression in lymph node metastases compared to primary tumours (7).

LRP has been identified as the major vault protein (MVP), the main component of multimeric vault particles. The exact role of LRP is still under investigation, although causality between LRP and drug resistance has been demonstrated (27,28). More recently in MVP (LRP) knockout mice the sensitivity of their bone marrow and stem cells to cytotoxic agents was studied. Based on these experiments their conclusion was that MVP/vaults had no direct role in resistance to chemotherapeutics (29). Analogous to P-gp and MRP, LRP expression has been studied as a potential predictor of response to chemotherapy (30). Whether LRP is up-regulated during the metastatic process of a tumour has not been investigated before. Previously we demonstrated in 141 primary STS, LRP expression in 74% (8), which is in line with the positivity of the present study including metastasised STS only. From the results of the current study, we conclude that LRP expression remains stable during the metastatic process in STS.

There are still some other issues to address in the interpretation of our results. In the present study the number of lymph node metastases was relatively high. The reason for this was merely clinical: lymph node metastases are removed relatively easily for both diagnosis and treatment. Radiographically overt pulmonary lesions in a patient with a high grade STS are highly suspect of metastatic progression. Rarely, such pulmonary lesions are removed with curative aim. This explains why the number of lung metastases in the present study is disproportionate to the clinical situation. Therefore, whether the findings of the current study are representative for the clinical situation remains to be shown in a larger cohort of STS patients with lung metastases.

Related to the high incidence of lymph node metastases in our study is the fact that certain histological types, such as angiosarcoma and rhabdomyosarcoma, were relatively over-represented. This does not reflect the common distribution of histological subtypes in STS (31).

The question of whether expression of multidrug resistance proteins in metastases increases after exposure to chemotherapy can only be answered in studies with adequate sample size. Given the fact that histological types of STS display different biological behaviour, this should ideally be done per single type. In a study dealing with rhabdomyosarcomas, chemotherapy appeared to up-regulate LRP expression (32). In this particular study, the majority of post-chemotherapy samples were taken from residual primary lesions; only two metastases were available,

both cases showing increased LRP expression compared to the chemotherapy naive primary. Obtaining material of a substantial number of pulmonary metastases, *i.e.* the commonest site of metastasis, is hampered by ethical constraints. In this respect, it is a pity that the European Organization for Research and Treatment of Cancer (EORTC) study STBSG 62933, a spin-off from the study by van Geel (33) and randomising between preoperative chemotherapy *versus* direct surgical removal of lung metastases, had to be stopped prematurely because of too slow patient accrual.

In conclusion, metastases of STS do not show a higher expression of multidrug resistance proteins P-gp, MRP1 and LRP than their primary counterparts. On the contrary: P-gp expression was lower in the group of metachronous metastases than in their primary tumours. This is in line with the observation that, also in case of metastatic disease of STS, doxorubicin still induces responses in 20-30% of the patients (34).

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