Acta Histochemica 116 (2014) 1390–1398

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

MRP1 and GSTp1 expression in non-small cell lung cancer does not correlate with clinicopathological parameters: A Slovakian population study

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ARTICLE INFO

Article history:
Received 9 July 2014
Received in revised form 2 September 2014
Accepted 3 September 2014

Keywords:
Lung cancer
MRP1
Glutathione-S-transferase p1
Immunohistochemistry

ABSTRACT

We detected MRP1 (multidrug resistance-associated protein 1) and GSTp1 (glutathione-S-transferase p1) protein expression in samples of non-small cell lung cancer (NSCLC) and our results were compared to basic clinicopathological parameters. The indirect immunohistochemical method was used for localization of monitored proteins. A total of 135 tissue samples of NSCLC were characterized according to histopathological type of tumor. Next, we compared our results with basic clinicopathological parameters (histopathological type of tumor, tumor grade and TNM stage of disease). In MRP1 and GSTp1 positive tumor cells, strong brown cytoplasmic immunostaining was visible. In our set of samples 71% showed MRP1 positivity, while according to histopathological type the squamous cell carcinoma reached the highest level of positivity (76%). Our GSTp1 results showed that similarly to MRP1, 70% of samples were GSTp1 positive. According to histopathological type the adenocarcinoma samples showed the highest GSTp1 expression (77%). For precise statistical evaluation the Kruskal–Wallis, Chi-square and Mann–Whitney tests were used. We did not find any statistically significant correlations between MRP1 and clinicopathological parameters. In the group of GSTp1, by Mann–Whitney test we found a statistically significant correlation between GSTp1 and histological grade ($p = 0.025$) in adenocarcinoma samples. As this was only one group of statistically significant correlation we wanted to confirm this finding. For this we applied also Chi-square test which revealed no statistically significant dependence ($p = 0.077$). No statistically significant relation was seen in the coexpression of both proteins ($p = 0.753$). Despite this, the majority of samples simultaneously expressed MRP1 and GSTp1 proteins. In conclusion, our results show that MRP1 and GSTp1 proteins represent independent prognostic features in NSCLC. Nevertheless, the clinical outcome in individual patients is often difficult to predict. Identification of the factors that characterize the resistant cases would permit immediate treatment of the patients with alternative therapeutic approaches.

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Introduction

According to statistical data, lung cancer in men in Slovakia is the leading cause of cancer death today. The number of new cases of lung cancer is about 2000 per year, with over 66% men and 90% of them were smokers.

Several factors contribute to the lethality of lung cancer, including the rapidity of tumor growth, advanced stage at diagnosis (due to non-specificity of early symptoms and the uncertain efficacy of screening), early development of metastases, and resistance to therapy.

There are several lung cancer histological types. The most frequent being the non-small cell lung carcinoma (NSCLC) with variants such as adenocarcinoma (45%), squamous cell carcinoma (30%) and large cell carcinoma (9%). All together NSCLC represents 85% of lung carcinomas. The small cell lung carcinoma (SCLC) constitutes approximately 15% of all types of lung cancers (Gazdar and Minna, 2004). An estimated 10–25% of lung cancers worldwide occur in non-smokers, defined as individuals having smoked less than 100 cigarettes in their lifetime. Lung cancer in non-smokers (LCINS) is more frequent in women, although large geographic variations are found. Histologically, adenocarcinomas predominate (Couraud et al., 2012). Until recently, most cases of NSCLC were

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http://dx.doi.org/10.1016/j.acthis.2014.09.002
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treated more or less as if they were the same disease. It has been postulated that common cancers are both common and resistant to therapy since many different mutations may give rise to them, and that each mutation may require a different treatment approach (Braiteh and Kurzrock, 2007; Dong et al., 2008).

Surgery remains the frontline therapy for stage I NSCLC. For patients with operable stage II and IIIA NSCLC, adjuvant systemic chemotherapy has been shown to improve 5-year survival by approximately 5% compared with surgery alone and has recently become the standard treatment. The current standard treatment for patients with advanced NSCLC is a doublet chemotherapy regimen, which commonly includes a platinum based drug (cisplatin or carboplatin) and gemcitabine (Yeh et al., 2005). The primary antitumor mechanism of platinum drugs is through the formation of platinum–DNA adducts, which if not repaired, lead to cell death. The major cellular pathways that control cisplatin action, and deficiencies in these pathways contribute to cisplatin resistance are: reduced drug accumulation, detoxification by thiol-containing species and related enzymes *e.g.*, GST-glutathione S transpherase – family proteins, increased repair of platinum–DNA adducts, decreased apoptosis and loss of mismatch repair (Cepeda et al., 2007; Kelland, 2007).

Detoxification of platinum drugs is mainly carried out by the glutathione detoxification system. The enzymes GSH (glutathione) peroxidase and GSH reductase may play a role in cisplatin resistance (Ogawa et al., 1993). GSTs (glutathione-S-transferases) catalyze the reaction of glutathione with drugs, yielding glutathione S-conjugates that are more water-soluble and less reactive than their parent compounds. The association between GST and chemotherapy resistance varied across studies. While results vary across cell lines, the bulk of available preclinical evidence suggests that GSH may play a role in resistance to cisplatin and perhaps other agents. In number of NSCLC (Kawai et al., 2002) but also in SCLC (Shellard et al., 1993) cell lines, increased GSTs content was associated with resistance to cisplatin (Kawai et al., 2002) with reduced platinum–DNA binding (Jain et al., 1996) and reduced intracellular platinum accumulation (Moritaka et al., 1998), accumulation of etoposide (Curtin and Turner, 1999), anthracyclines (Curtin and Turner, 1999), vinca alkaloids (Jain et al., 1996), camptothecins (Moritaka et al., 1998), mitomycin-C (Moritaka et al., 1998), alkylating agents (Moritaka et al., 1998), methotrexate (Moritaka et al., 1998), and radiation (Oshita et al., 1992). GST inhibitors increased sensitivity to cisplatin (Awasthi et al., 1994), and cisplatin resistance correlated with GST activity (Sharma et al., 1993) or with GST isoenzyme glutathione-S-transferase-p (GSTp) expression (Inoue et al., 1995) in NSCLC explants (Inoue et al., 1995), in some NSCLC cell lines (Hida et al., 1993), and in some SCLC cell lines (Awasthi et al., 1994).

The GSTs can operate in synergy with the efflux transporters. Specifically, it may bind and inactivate cisplatin (Meijer et al., 1990) and potentiate drug efflux *via* GS-X pumps mainly *via* efflux ABC transporter MRP – multidrug resistance-associated protein 1 (MRP1, ABCC1) (Zaman et al., 1995).

MRP1 is a member of ABC membrane transporter family proteins and confers resistance to some anticancer drugs, also platinum based drugs. Protein MRP1 or mRNA expression in lung cancer cell lines was associated with decreased accumulation, not only of cisplatin (Henness et al., 2002), but also of paclitaxel, anthracyclines (Henness et al., 2002), vinca alkaloids (Henness et al., 2002), etoposide (Young et al., 2001), taxanes (Oguri et al., 2008), gemcitabine (Oguri et al., 2006) and other agents (Gonzalez Manzano et al., 1996). The expression of MRP1 increased after exposure to platinum-based regimens suggesting that it may be upregulated as a protective response (Triller et al., 2006). High MRP expression was associated with decreased response rates (Triller et al., 2006; Ushijima et al., 2007) or survival (Yoh et al., 2004) in SCLC (Triller et al., 2006; Ushijima et al., 2007) and NSCLC (Yoh et al., 2004) patients receiving platinum-based combinations (Ushijima et al., 2007) or with vindesine plus etoposide (Oshika et al., 1998).

NSCLC cell lines tend to have greater expression of multidrug resistance protein (MRP) (which may function as a glutathione S-conjugate (GS-X) pump (Zaman et al., 1995) than do SCLC cell lines (Young et al., 2001). MRP3 (Young et al., 2001) and MRP7 (Oguri et al., 2008) may be particularly important. The impact of MRP on resistance may be decreased by 5-fluorouracil (Zhan and Liu, 1999) (5-FU), by verapamil, (Narasaki et al., 1997) or by glutathione depletion (Zaman et al., 1995). Clinically, MRP expression is common in both NSCLC (Xu et al., 1999) and SCLC (Campling et al., 1997). Clinical data in lung cancer remain limited and identification of the factors that characterize the resistant cases would permit immediate treatment of the patients with alternative therapeutic approaches.

Table 1
Patients and tumor characteristics.

Characteristics	No/%
All patients	135/100%
Histopathological type	
Adenocarcinoma	56/41%
Squamous cell carcinoma	58/43%
Large cell carcinoma	12/9%
Other types	9/7%
Histological grade	
G1	11/8%
G2	48/36%
G3	65/48%
G4	11/8%
TNM stage	
IA, IB	90/67%
IIA, IIB	19/14%
IIIA, IIIB, 4	26/19%

Material and methods

Patient samples and immunohistochemistry

The aim of our study was the immunohistochemical evaluation of the expression of MRP1 and GSTp1 proteins in 135 samples of NSCLC in relation to basic clinicopathological parameters such as: histopathological type, histological grade and TNM stage of disease. All samples were obtained from patients who underwent radical resection (lobectomy or pulmonectomy and lymphadectomy). The pathological diagnosis was based on WHO criteria (Beasley and Brambilla, 2005) and advanced stage was assessed according to TNM classification (Rami-Porta et al., 2009). The patients, who came from different regions of Slovakia, gave their informed consent before their inclusion into the study. The detailed characteristics of the study group are shown in Table 1.

Our NSCLC samples were divided according to histopathological type into: adenocarcinoma (56 samples), squamous cell carcinoma (58 samples), large cell carcinoma (12 samples), and other types (nine samples – small cell lung carcinoma, carcinoid). For immunohistochemical detection of proteins the following antibodies were used: polyclonal antibody anti-glutathione-S-transferase-pi, AB 8902, (Chemicon International, Temecula, CA, USA) for GST-p; and monoclonal antibody MRPm6, (Alexis Biochemicals, San Diego, CA, USA) for MRP1. To detect the bound GSTp1 antibody the Universal Detection Kit LSAB+KIT/HRP (Dako, Glostrup, Denmark) was used. For detection of MRP1 we have used the R.T.U. Vectastain Universal ABC Kit (Vector Laboratories, Burlingame, CA, USA.). Slides were exposed to 3,3′-diaminobenzidine (DAB) and counterstained with Mayer's hematoxylin. The negative controls were made by omitting

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