



Metallothionein Lower Under-Expression in Benign Tumors than That in Malignant Tumors: Systematic Review Article and Meta-Analysis

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Abstract

Metallothionein (MT) manifests varying expression levels in carcinomas, and they may be considered as valuable cell cancerization biomarkers for diagnosis of patients with cancers. A meta-analysis was conducted to evaluate comprehensively the MT expression difference in various benign tumors and malignant tumors, which compared the high with low MT expression levels in patients of the available studies. Finally, a total of 13 studies dealing with various tumors were involved for this meta-analysis. The results indicated that lower expression of MT in various benign tumors tissue than that in corresponding malignant tumors with the pooled OR of 0.52 (95 % CI 0.18-1.47, $P < 0.001$). In conclusion, MT expression difference is associated with tumor various stages in tumor patients and could be a useful clinical criteria of distinguishing benign tumors and malignant tumors for those patients.

Keyword: Metallothionein, Benign tumors, Malignant tumors, Meta-analysis

Introduction

In recent years, some studies about cancer are indicated that, when human's some tissues occur cancer, human's genes will appeared the abnormally expression of increase or decrease, we called these genes as cancer's marker gene or DNA methylation. DNA methylation, a common DNA epigenetic modification, can cause many human diseases by altering gene transcriptional machinery (1). In addition, such genes also have significance to predict the treatment result of cancer patients. For example, overexpression of Ki-67 antigen in the patients suggests their shorter survival time in comparison with normal/lower expression patients (2). Thus, if the marker gene or DNA met-

ylation expression abnormally, generally speaking, it will be proved that the cancer cells are destroyed the human tissues.

These arise various and abundant marker genes in human tissue, these marker ranges from conventional single protein-, RNA-, or DNA-based markers to molecular signatures built on multiplex assays, for instance, metastatic lymph nodes in the axilla (3-6), serum cytokeratin fragment 21-1 (CYFRA 21-1) (7, 8), estrogen receptor and human epidermal growth factor receptor 2 (HER2) (9, 10). More recent investigations have revealed that benefit from crizotinib (11) and anti-epidermal growth factor receptor antibodies (12, 13) re-

spectively, a marker gene is indicated by *ALK* translocations in lung cancer and the absence of *KRAS* mutations in colorectal cancers. Metallothionein (MT) is also among them, and plays an important role in all marker genes.

MTs are highly conserved, small molecular weight, cysteine rich proteins. They guard cells against the effects of heavy metals and from damage induced by free radicals (14). MT has been involved in several aspects of cancer pathobiology, for example, differentiation, proliferation, apoptosis and invasion (15). Conclusion is reached that immunohistochemically demonstrated MT expression is closely related to tumors stages (16). Furthermore, a major difference in MT expression was noted between different tumors stages even in the same tissue. For example, MT-III mRNA expression was remarkably higher in NSCLC (nonsmall-cell lung cancer) as against non-malignant lung tissues (17). The malignant gastrointestinal stromal tumors demonstrated higher MT-I/II expression as compared to the benign gastrointestinal stromal tumors, but not significantly higher (18). The levels of MT immunoreactivity were statistically appreciably higher in the tissue samples from squamous cell carcinoma than in those of the reference group and stromal samples (19). The MT expression level in gastrointestinal stromal tumor was confirmed to be significantly lower in comparison with gastric carcinoma tissues on the basis of the percentage stained and immunoreactive score (20). MT-expression in SCC (squamous cell carcinomas) (mean 2.89+/-1.83) was significantly higher than in actinic keratoses (mean 1.69+/-1.26) ($P=0.006$) and higher than in normal skin (mean 2+/-0.79) ($p=0.0075$) (21). Tumors showing incidental high metallothionein expression and negative p53 (metallothionein(H)/p53(-)) were significantly inversely related to depth of invasion, frequency of nodal metastasis, and Dukes stage ($P < 0.01$) (22). A significantly higher MT-I/II expression was noted in the grade 3 (G3) carcinomas as compared to those of G1 and G2 (23).

Therefore, metallothionein is supposed to be a useful biomarker linked to invasiveness, poor differentiation and malignant phenotype in tumor/cancer. However, the expression difference

of MT detected by immunohistochemistry in benign tumors and malignant tumors remain controversial, and the number of cases enrolled in numerous studies published was not largely enough. Therefore, we need to analyze the data of MT systematically in different tumor/cancer to draw a sensible conclusion about its expression differences in benign tumors and malignant tumors from different tissues/organ.

This study used a meta-analysis to explore MT expression differences in benign tumors and malignant tumors from different tissues/organs and what type of tumor has a lower MT expression level than the same tissue cancer.

Methods

Literature search

We searched the PubMed, EMBASE and Web of Science databases based on the terms: "metallothionein", "expression", "neoplasms", "Cancer", "prognosis", "benign", "malignant" with all possible combinations to identify relevant published papers from Jan 1st, 1981 to Dec 31, 2013. The references of all the studies were manually searched for additional eligible studies. We also inspected review articles and bibliographies of other pertinent articles to find related articles.

Inclusion and exclusion criteria

The inclusion criteria adopted for the meta-analysis included: 1) to evaluate metallothionein expression by immunohistochemistry in the human tumors and cancer tissues; 2) to assess the relationships between metallothionein expression level and tumor stages; 3) to be published in English language; 4) to provided sufficient information to estimate odds ratio (OR) and their 95% confidence intervals (CIs).

The articles were not in the scope of our analysis if they complied with these criteria: 1) letters, reviews, conference abstracts, case reports; 2) articles which do not offer enough data to calculate the OR about MT expression difference; 3) articles published in non- English; 4) overlapping articles (Fig. 1).

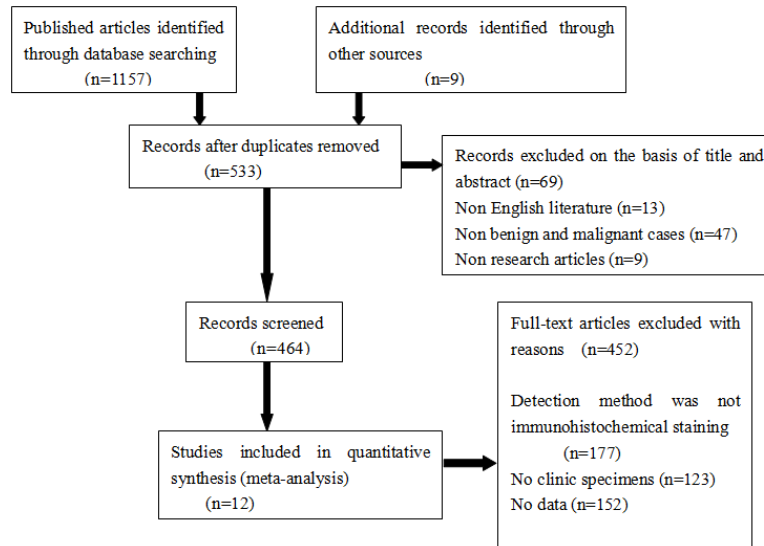


Fig. 1: Flow diagram of the study selection process

Data extraction and assessment of study quality

Two investigators (Ruijie Sun and Mengmeng Liu) reviewed each eligible study and extracted following data: the name of the first author, publication year, nationality, number of patients, number of benign tumor patients, number of malignant tumor patients, tumor site and antibody source.

Controversies were left to the arbitration of the third investigator (Jie Zhang). Newcastle-Ottawa quality assessment scale was employed to evaluate the quality of each study (24). Finally we selected 12 articles, and the statistics of the benign and malignant numbers are respectively 333 and 569 (Fig. 2A).

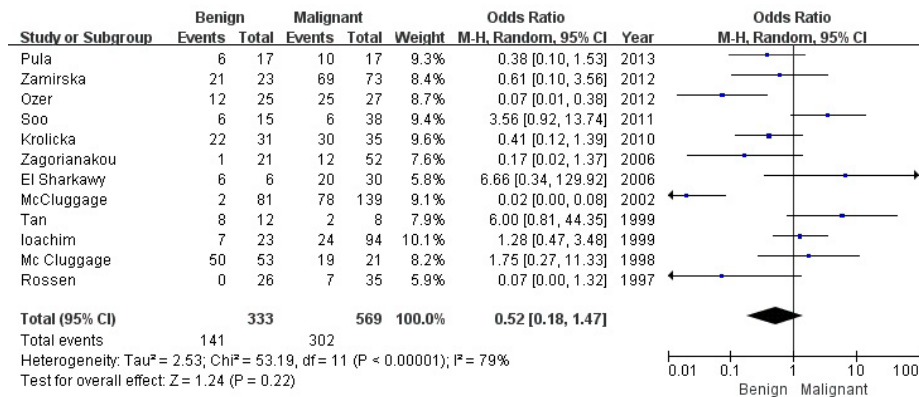


Fig. 2 A: Forrest plot of odds ratios (ORs) with corresponding 95% CIs of the MT expression in both benign and malignant tumors. OR<1 suggested that lower expression of MT in various benign tumors tissue than that in corresponding malignant tumors

Statistical analysis

Odds ratios (ORs) and their 95% CIs were combined to assess the link between metallothionein expression and clinicopathological factors, such as, the occurrence of different parts of human’s body, differentiation grade, Dukes’ stages, depth of invasion, lymphnode status and metastasis. For

the pooled analysis of metallothionein expression difference in benign tumors and malignant tumors from different tissues/organs, ORs and its 95% CIs were the advocated summary statistics for meta-analysis of MT in different tumor stages. If these statistical variables were described in a literature, we pooled it directly; otherwise, they were calcu-

lated from available numerical data in the articles according to the methods described by Parmar (25). An observed OR=1 implies unfavorable parameters for the group with metallothionein expression difference in benign tumors and malignant tumors from different tissues/organs. The impact of increased metallothionein expression on different tumor stages was thought to be statistically significant if the 95% CIs did not overlap with 1. Heterogeneity across studies was assessed by Chi-square based Q statistical test. And the I^2 statistic to quantify the total variation proportion, which is ascribed to inter study heterogeneity instead of sampling error and is measured from 0% to 100%. A $P > 0.10$ for the Q-test indicated an absence of heterogeneity among the studies, then the pooled ORs estimate of each study were calculated by the random-effects model (the Mantel-Haenszel method). In other cases, the model of random-effects (the Der Simonian and Laird method) was used. Funnel plot was used to evaluate the probability of publication bias. The statistical analyses were carried out using Review Manager 5 software (The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark). All the P values were for a two-side test and considered statistically significant when $P < 0.05$.

Results

Description of studies

Totally 1157 studies were identified from a search of the above databases using the search strategy as described above (Fig. 1). A scrutiny of the abstracts and full-text of these studies showed that a total of 12 eligible studies were ultimately chosen in this meta-analysis (17, 20, 21, 26-34). The clinical features of these 12 included studies were summarized in Table 1. These studies were published from 1997 to 2013, and total 902 cancer patients, of which include various benign tumors and malignant tumors, were enrolled and investigated the MT expression difference between benign tumor and malignant tumor from various tissues/organs. Sample sizes ranged from 20 to 220 patients. 10 studies enlisted less than 100 pa-

tients and 2 studies involved more than 100 patients. Of these 12 studies, 3 studies were conducted in Poland, 2 each in Singapore, Northern Ireland and Greece, 1 each in Egypt, Denmark and Turkey.

Meta-analysis

For studies evaluating metallothionein expression in benign tumors and malignant tumors, there was obvious between-study heterogeneity among those 12 studies for metallothionein ($I^2=79\%$) (Fig. 2A), so the random-effect model and subgroup meta-analysis were used to calculate the pooled OR with corresponding 95 % CIs. We investigate each factor, including the test method, publication country, number of patient and the data extraction method, which contributed to the heterogeneity. The results showed that the meta-analysis is vigorously influenced obviously by 4 papers' data which their data do not directly appear in the paper. So the 4 papers' data, that is numbers of patients, were obtained according to inference of other data in the paper. For example, if these data were removed, the analysis results changed significantly with low heterogeneity between remained studies ($\chi^2=13.64$ ($P=0.06$) and $I^2=49\%$). No matter before excluding 4 papers or after, the meta-analysis results all indicated that there is a lower metallothionein expression in benign tumors than that in malignant cancers. This conclusion may be associated with differential expression outcome in various tumors/carcinomas stages, with the pooled OR of 0.52 (95 % CI 0.18-1.47, $P < 0.00001$) or 0.68 (95 % CI 0.41-1.10, $P=0.12$) in subgroup (Fig. 2A & Fig. 2C). The tissues analyzed in our meta-analysis include gastrointestinal, ovarian, skin sections, thyroid follicular, epithelial ovarian, prostatic, colorectal, cervical and dermatofibromas et al.

Publication bias

Funnel plot were used to determine the publication bias of the literatures. The shape of the funnel plot did not suggest any proof of obvious asymmetry (Fig. 2B & Fig. 2D).

Table 1: Characteristics of studies included for the meta-analysis

References	Country	Patient (m/b)*	Antibody source	Tumor site	Benign Overexpression oe/b(%)*	Malignant overexpression oe/b(%)*
(17)	Poland	34(17/17)	monoclonal antibodies(DakoCytomation Glostrup, Denmark)	Gastrointestinal	6/17 (35.3)	10/17 (58.8)
(26)	Turkey	52 (27/25)	Mouse monoclonal, CloneE9 (Neomarkers, USA)	Ovarian	12/25(48)	25/27 (92.6)
(21)	Poland	96(73/23)	Ki-67 antigen (Dako, Denmark)	Skin sections	21/23(92)	69/73(95)
(20)	Singapore	53 (38/15)	Primary mouse anti-horse antibody E9 ((Dako)	Gastric	6/15(40)	6/38(15.9)
(27)	Poland	66(35/31)	monoclonal antibodiesby DakoCytomation (Glostrup, Denmark)	Thyroid follicular	22/31 (70.9)	30/35 (85.7)
(28)	Greece	73(52/21)	E9 (Dako)	Epithelial ovarian	1/21 (4.8)	12/52(23.9)
(29)	Egypt	36 (30/6)	E9 (Dako Corporation, Glostrup, Denmark)	Prostatic	6/6(100)	20 /30(66.7)
(30)	Northern Ireland	220(139/81)	Monoclonal antibody E9 (DaKo,Ely,UK)	Epithelial ovarian	2/81(2)	78/139(56)
(32)	Greece	117 (94/23)	monoclonal antibodies against Ki-67 (Dako)	Colorectal	7/23(30)	24/94(25)
(31)	Singapore	20(8/12)	primary antibody E9 (Dako, Copenhagen, Denmark)	Ovarian	8/12(66.7)	2/8(25)
(33)	Northern Ireland	74 (21/53)	Monoclonal antibody E9 (Dako, Copenhagen, Denmark).	Cervical	50/53(94)	19/21(90)
(34)	Denmark	61(35/26)	MIBI(Immunotche Maresilles ,France) mouse monoclonal IgCl antibody E9 (clone E9. DAKO, Denmark)	Dermatofibromas	0/26(0)	7/35(20)

*b: Benign; m: Malignant; oe: Overexpression

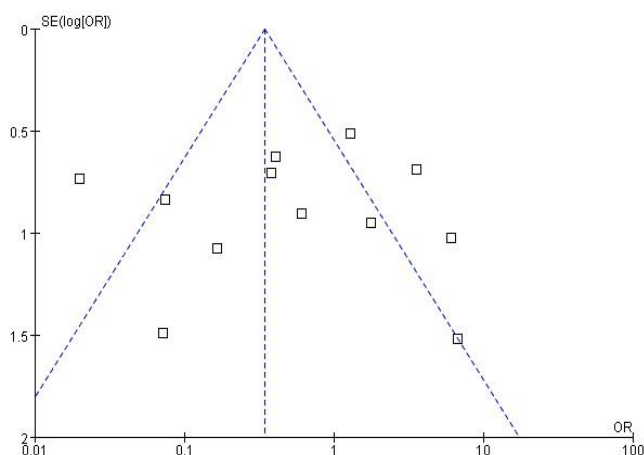


Fig. 2B: Funnel plot for the publication bias

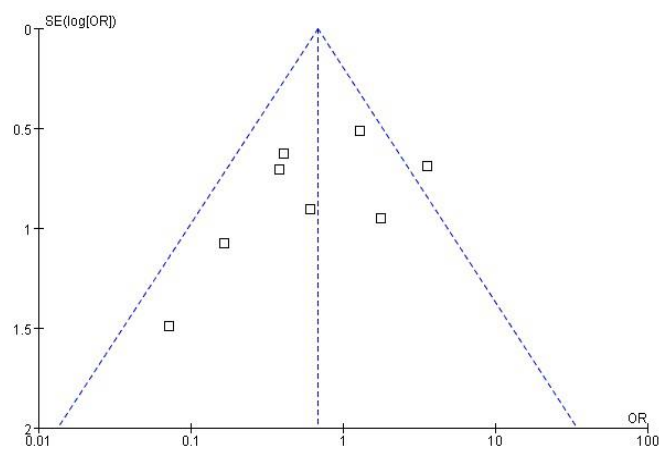


Fig. 2D: Funnel plot for the publication bias

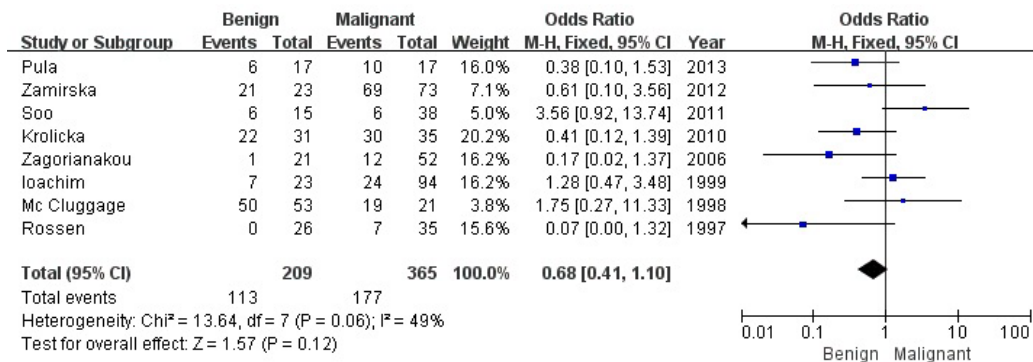


Fig. 2C: Forrest plot of odds ratios (ORs) with corresponding 95% CIs of the MT expression in both benign and malignant tumors

Discussion

MT, a group of low-molecular-weight (6-7 kDa) intracellular metal-binding proteins expressed in many tissues (35), plays a vital role in apoptosis and proliferation, which can exert an influence on the carcinogenic process. However, there is no consensus on the MT expression difference in benign tumors and malignant tumors from different tissues/organ at present. Meta-analysis is a systematical approach applied widely to the evaluation of gene expression difference in different trials. Thus, a meta-analysis was conducted to determine the characteristic of MT expression difference in benign tumors and malignant tumors from different tissues/organ.

The study aimed at comparing the MT lower under-expression in the benign than the malignant by meta-analysis. And the statistical results have already displayed in the Fig.2A. Fig.2A showed MT expression differently in benign tumors and malignant tumors from different tissues/organs. Moreover, there is a lower metallothionein expression in benign tumors than malignant cancers.

The results disclosed the higher MT expression associated with malignant. MT as a representative of an important cancer's marker gene has very important significance in the future clinical work. Especially, in the assessment for the differential diagnosis of benign and malignant tumors, and evaluated the cancer patients' treatment result. Previous many studies found that the MT expression level in tumor or cancer was up-regulated,

however the comparative analysis of MT expression change in tumor various stages has not been established. To explore the difference of MT expression in tumor various stages (especially benign tumors and malignant tumors), our analysis combined the outcomes of 12 studies comprising 902 cancer patients, indicating that there is a lower metallothionein expression in benign tumors than that in malignant cancers (OR=0.52, 95% CI 0.18-1.47, $P < 0.00001$). In this meta-analysis, we had dealt with highly significant heterogeneity among the 12 studies. Although random effects models were used to analyze the data, heterogeneity was still a possible problem to affect meta-analysis results. So we only selected studies with methods of immunohistochemistry to lower heterogeneity as soon as possible, but source and dilutions of primary antibodies, evaluation standards (such as patients' number or immunohistochemistry staining score), study location, number of patients, sex and age of patients were quite different, which contributed to the heterogeneity inevitably. When the analysis on the difference of MT expression in both benign and malignant tumors was performed without consideration of other factors, obvious heterogeneity was identified ($I^2 = 79\%$). When 4 papers' data were removed from data queue, the heterogeneity significantly reduce ($I^2 = 49\%$). At the same time, this meta-analysis was limited in some ways. First, the study covered by our meta-analysis was confined only to articles published in English, which probably brought about extra bias. Second, previous some

studies showed that there was a lower expression in tumor tissue than that in normal tissue. For instance, the most frequent molecular events connected to ependymoma recurrence were over-expression of kinetochore proteins and down-regulation of metallothioneins (36). Immunohistochemical evaluation of all metallothionein I/II isoforms in colorectal tumors has borne out an important down-regulation in comparison with normal tissue (37). MT expression drops with the degree of histological differentiation and drops with increasing tumor stage in hepatocellular carcinoma (38). 75/112 (67%) gastric carcinomas showed MT immunoreactivity with a significant lower expression in advanced gastric carcinomas (39). But these studies all were excluded because they failed to meet our criteria.

Conclusion

Despite of the limitations mentioned above, the current evidence suggests that the immunohistochemical evaluation of MT in benign tumors has revealed an important down-regulation in comparison with malignant tumors, this MT expression difference appeared in gastrointestinal, ovarian, skin sections, thyroid follicular, adrenal cortex, epithelial ovarian, prostatic, breast, colorectal, cervical and dermal tumor, respectively. However, larger-scale prospective studies are needed in future to further confirm our results.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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