EXPERIMENTAL CELL RESEARCH ▮ (▮▮▮▮) ▮▮▮–▮▮▮

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Research Article

Tetraspanin 7 (TSPAN7) expression is upregulated in multiple myeloma patients and inhibits myeloma tumour development *in vivo*

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ARTICLE INFORMATION

Article Chronology:
Received 25 July 2014
Received in revised form
13 January 2015
Accepted 16 January 2015

Keywords:
Multiple myeloma
Tetraspanin 7
Calnexin
Migration
Adhesion

ABSTRACT

Background: Increased expression of the tetraspanin TSPAN7 has been observed in a number of cancers; however, it is unclear how TSPAN7 plays a role in cancer progression.
Methods: We investigated the expression of TSPAN7 in the haematological malignancy multiple myeloma (MM) and assessed the consequences of TSPAN7 expression in the adhesion, migration and growth of MM plasma cells (PC) *in vitro* and in bone marrow (BM) homing and tumour growth *in vivo*. Finally, we characterised the association of TSPAN7 with cell surface partner molecules *in vitro*.
Results: TSPAN7 was found to be highly expressed at the RNA and protein level in $CD138^+$ MM PC from approximately 50% of MM patients. TSPAN7 overexpression in the murine myeloma cell line 5TGM1 significantly reduced tumour burden in 5TGM1/KaLwRij mice 4 weeks after intravenous adminstration of 5TGM1 cells. While TSPAN7 overexpression did not affect cell proliferation *in vitro*, TSPAN7 increased 5TGM1 cell adhesion to BM stromal cells and transendothelial migration. In addition, TSPAN7 was found to associate with the molecular chaperone calnexin on the cell surface.
Conclusion: These results suggest that elevated TSPAN7 may be associated with better outcomes for up to 50% of MM patients.

Abbreviations: ALL, acute lymphoblastic leukaemia; BM, bone marrow; BMEC, BM endothelial cell; BMSC, bone marrow stromal cells; BMMNC, BM mononuclear cells; LC, liquid chromatography; MFI, median fluorescence intensity; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; MS/MS, mass spectrometry; PC, plasma cells; PCL, plasma cell leukaemia; SDF-1α, stromal cell derived factor-1α; SMM, smouldering MM

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http://dx.doi.org/10.1016/j.yexcr.2015.01.006
0014-4827/

Introduction

Multiple myeloma (MM) is an incurable haematological cancer characterised by the uncontrolled proliferation of clonal malignant plasma cells (PC) within the bone marrow (BM). MM is the second most common haematological cancer after non-Hodgkin's lymphoma. Despite recent treatment advances, MM remains universally fatal with a 10-year survival rate of ~17% [8]. The main clinical features of MM are osteolytic bone lesions, hypercalcaemia, renal insufficiency, suppressed immunoglobulin production and increased BM angiogenesis. In most patients, active MM is preceded by the benign condition monoclonal gammopathy of uncertain significance (MGUS) [39,77].

In most MM patients at diagnosis, malignant PC are not only found at the initial site of BM establishment, but have disseminated to multiple sites in BM. One likely mechanism of MM tumour dissemination is via the peripheral circulation, in a similar mode to that utilised by solid tumours. As malignant PC are derived from B lymphocytes, the dissemination of MM may adopt similar extravasation and homing mechanisms to those used in haematopoietic cell trafficking [23]. Additionally, increasing evidence suggests that MM PC employ mechanisms involved in the metastasis of solid tumours in order to spread [23]. For example, the homing of the MM PC to the BM is stimulated by chemoattractants, including stromal cell derived factor-1α (SDF-1α/CXCL12), that are produced by BM stromal cells (BMSC) [52]. In response to these signals, the PC adhere to BM endothelial cells via specific receptors, secrete proteases including matrix metalloproteinase 9 and migrate across the endothelium into the extravascular marrow space [74].

The ability of tumour cells to invade and metastasise is regulated by a number of cell surface proteins. Tetraspanins are transmembrane 4 superfamily (TM4SF) proteins that are unique in their capacity to associate laterally with other membrane proteins such as integrins, immunoglobulin superfamily members, major histocompatibility complex molecules and signalling receptors and with intracellular signalling proteins to form lipid-rich membrane domains known as tetraspanin-enriched microdomains [82,85]. By acting as membrane organisers, clustering partner proteins into functional complexes, tetraspanins modulate a range of cellular activities including adhesion and migration [91]. Dysregulation of tetraspanin expression has been observed in cancers of the breast, colon, bladder, liver, lung, cervix and ovary, with decreased expression of tetraspanins TSPAN8, CD9 (TSPAN29), CD81 (TSPAN28) and CD82 (TSPAN27) being associated with higher grade or metastatic tumours and disease recurrence [1,24,28,30,32,45,46,48,65,69]. While tetraspanins have been shown to have little or no effect on cancer cell proliferation or survival *in vitro*, tetraspanin overexpression increases adhesion and decreases migration and invasion in cancer cell lines *in vitro* and inhibits metastatic spread *in vivo* [17,35,42,58,59,68,71,83]. In myeloma, decreased expression of *CD9* and *CD63* has been associated with increasing disease stage in MM patients [4,15] while decreased expression of *CD9* has been associated with disease progression in the 5T33MM and 5T2MM mouse models *in vivo* [14,15]. CD81 is expressed in approximately 50% of MM patients, with decreased expression in MM when compared with normal BM PC [56,72]. Importantly, expression of cell surface CD81 predicts poorer progression-free survival and overall survival in patients with active MM, and more rapid progression from smouldering to active MM [72].

TSPAN7 (also known as CD231, TALLA-1, TM4SF2) is another TM4SF member which has been implicated in the development and progression of several cancers. TSPAN7 is broadly expressed in non-hematopoietic cells, with the greatest expression in the brain [29,87]. TSPAN7 was first described as being strongly expressed in T-cell acute lymphoblastic leukaemia (ALL) and in some neuroblastoma cell lines, but not in a panel of other adult T-cell leukaemia, B-cell-ALL, Burkitt's lymphoma, myelomonocytoid or solid tumour cell lines [70]. Subsequently, microarray analyses have demonstrated elevated *TSPAN7* expression in cancer of the stomach, pancreas, liver, oesophagus, colorectum [11,80,81]. In clear-cell renal cell carcinoma, increased TSPAN7 expression in primary tumour cells, by histology, is not associated with patient outcomes [81]; however, increased *TSPAN7* gene expression in clear-cell renal cell carcinoma lung metastases is associated with a longer metastasis-free interval [80]. Notably, *TSPAN7* expression is induced by transforming chimaeric transcription factors EWS-WT1(-KTS) and AML1/MTG8, suggesting a role in oncogenic transformation [20,34]. Collectively, these studies suggest that TSPAN7 expression is associated with carcinogenesis; however, the precise role of TSPAN7 expression in cancer has not been defined.

While loss of expression of other tetraspanins, including CD9 and CD82, has been implicated in MM development, the role of TSPAN7 in MM has not been investigated. In this study, we investigated the expression of TSPAN7 in primary MM PC and assessed the functional consequences of TSPAN7 expression in the adhesion, migration and growth of MM PC *in vitro*. In addition, we investigated the involvement of TSPAN7 in BM homing and intramedullary tumour growth in the 5TGM1/KaLwRij systemic myeloma model. Finally, we characterised the association of TSPAN7 with cell surface partner molecules in a MM cell line.

Materials and methods

Microarray analysis

For analysis of TSPAN7 and CNX expression in CD138-selected BM plasma cells from newly diagnosed MGUS, smouldering MM (SMM), MM, plasma cell leukaemia (PCL) patients or normal controls, three independent microarray datasets were used: E-GEOD-6477 (normal, $n=15$; MGUS, $n=22$; SMM, $n=24$; MM, $n=73$) [12] and E-GEOD-16122 (normal, $n=5$; MGUS, $n=11$; MM, $n=133$; PCL, $n=9$) [2], both on Affymetrix GeneChip Human Genome U133A arrays, and E-MTAB-363 (normal, $n=5$; MGUS, $n=5$; MM, $n=156$) [61] conducted on Affymetrix GeneChip Human Genome U133 plus 2.0 arrays. Analysis of *TSPAN7* expression in different patient subsets was conducted on datasets E-GEOD-19784 [9] and GSE4581 [88], both on Affymetrix GeneChip Human Genome U133 plus 2.0 arrays. Analysis of patient survival in *TSPAN7* high and *TSPAN7* low newly-diagnosed MM patients was carried out using GSE4581, analysing patients included in the total therapy 2 (TT2) trial. For all datasets except GSE4581, .CEL files were downloaded from ArrayExpress (EMBL-EBI) [64], files were normalised using RMA and $\log_2$ transformed in R (version 3.0.3). One MM patient in E-MTAB-363 (V0681) failed quality control (normalised unscaled standard error [NUSE] > 1.05) and so was excluded and the remaining 155 MM and 5 normal control files were re-normalised. For GSE4581, MAS5-normalised files

Please cite this article as: C.M. Cheong, et al., Tetraspanin 7 (TSPAN7) expression is upregulated in multiple myeloma patients and inhibits myeloma tumour development *in vivo*, Exp Cell Res (2015), http://dx.doi.org/10.1016/j.yexcr.2015.01.006