Critical Reviews in Oncology/Hematology 105 (2016) 127–144

Contents lists available at ScienceDirect

Critical Reviews in Oncology/Hematology

journal homepage: www.elsevier.com/locate/critrevonc

# Clinical significance of immunohistochemically detected extracellular matrix proteins and their spatial distribution in primary cancer

B. Čunderlíková [a,b,*]

[a] Faculty of Medicine, Comenius University, Bratislava, Slovakia
[b] International Laser Centre, Bratislava, Slovakia

**Contents**



ARTICLE INFO

*Article history:*
Received 8 May 2015
Received in revised form 3 April 2016
Accepted 27 April 2016

ABSTRACT

Our understanding of cancer has evolved mainly from results of studies utilizing experimental models. Simplification inherent to *in vitro* cell culture models enabled potential ways of cell behaviour in response to various external stimuli to be described, but it has led also to disappointments in clinical trials, presumably due to the lack of crucial tissue components, including extracellular matrix (ECM).

*Abbreviations:* 2D, two-dimensional; 3D, three-dimensional; APC, adenomatous polyposis coli; BCC, basal cell carcinoma; BM, basement membrane; CEA, carcinoembryonic antigen; CIS, carcinoma *in situ*; CNS, central nervous system; ECM, extracellular matrix; IHC, immunohistochemical; LOX, lysyl oxidase; MMP, matrix metalloproteinase; NSCLC, non-small cell lung carcinoma; PCNA, proliferating cell nuclear antigen; SCC, squamous cell carcinoma; SCLC, small cell lung carcinoma; SHG, second harmonic generation; SPARC, secreted protein acidic and rich in cysteine (osteonectin); SPARCL1, secreted protein acidic and rich in cysteine-like 1; TACS, tumour associated collagen signatures; TIMP, tissue inhibitor of metalloproteinases.

* Corresponding author at: Faculty of Medicine, Comenius University, Bratislava, Slovakia.
*E-mail address:* beata@ilc.sk

http://dx.doi.org/10.1016/j.critrevonc.2016.04.017

*Keywords:*
Extracellular matrix
Neoplasms
Patient outcome
Immunohistochemistry
Cell-matrix adhesion
Immune infiltrate

ECM and its role in healthy and diseased tissues are being explored extensively and significance of ECM for cell behaviour has been evidenced experimentally. Part of the information gathered in such research that is relevant for natural conditions of a human body can be identified by carefully designed analyses of human tissue samples. This review summarizes published information on clinical significance of ECM in cancer and examines whether effects of ECM on cell behaviour evidenced *in vitro*, could be supported by clinically based data acquired from analysis of tissue samples. Based on current approaches of clinical immunohistochemical analyses, impact of ECM components on tumour cell behaviour is vague. Except of traditionally considered limitations, other reasons may include lack of stratification of analyzed cases based on clinicopathologic parameters, inclusion of patients treated postoperatively by different treatments or neglecting complexity of interactions among tumour constituents. Nevertheless, reliable immunohistochemical studies represent a source of crucial information for design of tumour models comprising ECM corresponding to real clinical situation. Knowledge gathered from such immunohistochemical studies combined with achievements in tissue engineering hold promise for reversal of the unfavourable trends in the current translational oncologic research.



## 1. Introduction

Our understanding of processes and mechanisms related to cancer has evolved mainly from results of studies utilizing experimental models. Feasibility of cell culturing has constituted a powerful tool for data acquisition in biological research. For the most part, cell survival has become the only requirement for *in vitro* studies, whereas the question of whether cultured cells behave in a way similar to the one effective *in vivo* has been largely neglected. In words of mathematical analysis, however, cell survival is necessary but not sufficient condition for studies aimed at understanding biological processes. Simplification inherent to *in vitro* cell culture models enabled potential ways of cell behaviour in response to various biochemical and physical stimuli to be analyzed and described. Nevertheless, there is growing indication that the same simplification has led to many disappointments as well. This is not surprising taking into consideration many limitations of *in vitro* culturing (reviewed in Čunderlíková, 2013), including incompleteness of the models established to mimic systems with dynamic and reciprocal interactions among multitude of constituents (including those originating from the host (e.g. Iacobuzio-Donahue et al., 2002)), resulting in potential lack of factors that may shift processes to follow pathways with completely different or even opposite consequences compared to those effective under oversimplified conditions. Impact of some of these limitations has been evidenced even by simple modifications of the most conventional *in vitro* models by replacing 2D cell monolayers with more *in vivo* mimicking models, namely by cell co-cultures and 3D cell cultures (references in Čunderlíková, 2013). Use of animal models is a further step towards reality and many *in vitro* studies are now accompanied by *in vivo* experiments. Nevertheless, this is still hardly sufficient for direct extrapolation of experimental results to clinical situation. Thus, significance of species differences (Rettig et al., 1992; Virtanen et al., 2000; Predina et al., 2013, references in Čunderlíková, 2013; Perreault et al., 1998; Peddareddigari et al., 2010) is usually not addressed in experimental studies and animal tumour models may not fully represent human tumours (Dvorak, 1986; Koutroulis et al., 2008; Hutchinson and Kirk, 2011). Furthermore, functional studies in majority cases employ models where single gene is affected, although systems biology approaches identify whole signatures, i.e. groups of several genes or proteins with concomitantly altered expressions that distinguish individual biological entities. The consequences of affecting several genes at the same time are not so frequently explored experimentally and potential compensatory effects, such as those documented e.g. for integrins (references in van der Flier and Sonnenberg, 2001; Ekblom et al., 2003), or other additive, synergistic or antagonistic effects indicated e.g. for combination of extracellular (ECM) proteins (Reticker-Flynn et al., 2012) are not always considered.

The neglecting of the limitations of model systems may translate into lack of success in clinical trials, which we experienced especially in anticancer drug development (Hutchinson and Kirk, 2011; Thomas, 2012) that included development of agents directed against ECM-related targets (Nelson et al., 2000; Martin et al., 2008; Sun et al., 2014, references in Adams and Lawler, 2011). Such experience implies that cancer research based on experimental models should not only rely on generation of hypothesis for potential mechanisms, but confrontation with clinical knowledge to identify relevant information should represent critical part of it.

ECM and its role in healthy and diseased tissues are being explored extensively and significance of ECM for cell behaviour has been evidenced under experimental settings. Which part of the information gathered so far is relevant for natural conditions of a human body is an issue that needs to be addressed. This can be accomplished by carefully designed analyses of human tissue samples.

The purpose of this summary, focused mainly on primary tumours of epithelial origin, was to review published information on clinical significance of immunohistochemically (IHC) detected ECM in terms of associations between expressions of ECM proteins and clinicopathological parameters and/or patient outcomes. Additional intention was to examine, whether effects of ECM on cell behaviour evidenced *in vitro*, could be supported by clinically based data acquired from analysis of tissue samples – i.e. of complex systems consisting of mutually interacting and competing components.

## 2. ECM—current concept

Cells in tissues are surrounded by extracellular matrix (ECM) – a network of extracellular molecules, such as fibrillar and non-fibrillar proteins, glycoproteins and polysaccharides (Brown, 2011; Hynes and Naba, 2012; Triulzi et al., 2013) that are produced by cells, often as a result of specific cell–cell interactions (Frantz et al., 2010; Lu et al., 2012; Apte et al., 2004; Erkan et al., 2008; Bachem et al., 2005). Structure and function of ECM differ spatially in each tissue. As a result, different roles for ECM in tissues are being recognized depending on matrix composition, structure and location (Table 1).

The extent to which individual cells within tissue interact with ECM varies for different cell types. Stromal cells are completely embedded within ECMs and interact with ECM components without need for polarized expression of corresponding ECM adhesion receptors. In contrast, within multilayered stratified epithelium,