Short Communication

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The relationship between expression of *VIMENTIN* and *CD146* genes in breast cancer

https://doi.org/10.1515/bams-2020-0058
Received October 5, 2020; accepted February 25, 2021;
published online March 25, 2021

Abstract

Objectives: CD146 is an adhesive molecule that was originally reported on malignant melanoma cells as a protein crucial for cell adhesion. It is now known that high expression of the CD146 protein is not only characteristic of melanoma, but it occurs on a number of cancers, contributing to worse prognosis and increased aggressiveness. Independent *in vitro* studies in breast cancer have shown that CD146 protein alone can induce a change in epithelial to mesenchymal transcriptional profile, which is the basis of the tumor aggressiveness and metastasis.
Methods: In the following work, the correlation coefficients were analyzed between the genes of the mesenchymal profile and the *CD146* gene in 10 independent transcriptomic data of breast cancer patients.
Results: The analysis confirmed the relationship between *CD146* expression and mesenchymal profile genes, pointing *VIMENTIN* as the gene which expression is most strongly correlated with the *CD146*, suggesting that both genes, *CD146* and *VIM* may be directly controlled by the same mechanism or regulate one another.
Conclusions: The analysis points a potential route for research on the *CD146* gene expression, which may lead to understanding of its regulation in breast cancer, contributing to the development of new therapeutic strategies targeting highly metastatic breast cancer cells.

Keywords: *CD146 (MCAM, MUC-18)*; EMT; *Vimentin*.

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Introduction

CD146 was initially described on melanoma cells as an adhesion molecule contributing to the invasiveness of cancer cells. Of note, the currently published studies have reported the involvement of CD146 in progression and poor prognosis of several cancers, including breast cancer [1]. Interestingly, *in vitro* and *in vivo* data determined that CD146 introduction into breast cancer cells with epithelial characteristics trigger the changes in transcriptional profile leading to epithelial to mesenchymal transition (EMT) [2, 3]. The mesenchymal cancer cells are characterized by poor cell–cell adhesive interaction and increased migratory and invasiveness potential, which altogether leads to enhanced aggressiveness and promote the progression of the disease. A key event in the epithelial–mesenchymal transition is the change in gene expression profile from epithelial to mesenchymal [4, 5]. Although based on *in vitro* experiments, there is no doubt that overexpressed *CD146* contributes to the aggressiveness of tumors by promoting cadherin switch, mesenchymal profile and malignant cell motility, still little is known about the regulation of this gene in cancer cells. So far none of the published study reported translocation, amplification or mutation in *CD146* gene sequence in cancer cells [6]. From the other site, our preliminary data strongly suggest that epigenetic mechanism, exactly DNA methylation, may play a crucial role in the regulation of *CD146* expression in breast and prostate tumors [7–9]. Regardless of the mechanism regulating the expression of CD146, the research data indicates that overexpression of this protein *in vitro* triggers signaling to lead to an alteration in the epithelial expression profile into the mesenchymal one in breast cancer cells [2]. In line with this observation, we show here that *CD146* expression is significantly correlated with mesenchymal markers in several independent breast cancer patient's transcriptomic datasets. The analysis of the correlation coefficients performed in the current study showed that the *VIMENTIN* gene is the most strongly correlated mesenchymal marker with *CD146*, which may indicate that these two genes are regulated directly by the same overriding mechanism or regulate one another, which may be crucial for the

Table 1: The mean log2 expression value for analyzed mesenchymal markers in 10 breast cancer patients' datasets.

GEO ID	GSE7390	GSE46563	GSE1456	GSE12276	GSE2109	GSE3494	GSE10248	GSE29271	GSE69031	GSE36771
TWIST1	5.56	7.81	5.74	6.67	7.01	5.51	7.28	6.59	6.36	7.05
SNAI1	3.89	6.18	4.18	4.36	4.19	3.70	3.17	4.50	2.07	3.85
SNAI2	7.41	8.96	7.48	8.08	8.28	6.91	8.12	8.14	6.81	8.01
ZEB1	7.02	6.69	7.32	8.29	8.29	7.55	8.64	8.28	7.56	8.78
ZEB2	4.16	7.96	5.27	6.28	6.83	4.45	7.48	6.17	5.44	6.82
CDH2	4.35	8.04	4.8	5.00	5.15	3.92	5.47	4.95	4.52	3.78
VIM	11.52	13.23	11.38	12.10	12.40	11.35	11.86	12.17	10.69	12.27
MMP2	8.53	7.15	9.94	9.82	9.91	9.97	9.85	9.98	9.30	10.46
MMP9	7.76	10.02	8.02	8.42	8.91	8.27	8.49	8.51	8.29	8.54
FN1	12.00	6.45	11.92	12.73	12.87	11.72	12.49	12.73	12.81	12.81
CD146	7.09	6.31	7.51	7.63	7.85	7.57	7.57	7.66	7.86	7.86

initiation of the epithelial–mesenchymal transition. Our analysis indicates the direction for *in vitro* research, the results of which can significantly broaden our understanding of breast cancer progression.

Data and statistical analysis

To analyze the relation between *CD146* and mesenchymal markers, we used R2 database developed at the Department of Oncogenomics in the Academic Medical Center (AMC) in Amsterdam, Netherlands (http://r2.amc.nl). In the following study, we applied a tool "Correlate Genes" in the panel of 10 breast cancer patients transcriptomic datasets (GSE7390-u133p2 and GSE46563-ilmnhwg6v3, composed of only lymph node-negative patients and eight datasets GSE1456-u133a, GSE12276-u133p2, GSE2109-u133p2, GSE3494-u133a, GSE102484-u133a, GSE29271-u133p2, GSE69031-u133a, GSE36771-u133a), where patients were not selected

Table 1A: Spearman's rank coefficients for CD146 and 11 mesenchymal markers genes in 10 breast cancer patients' transcriptomic datasets. For significant positive correlations the R value is marked in gray.

GEO ID	GSE7390		GSE46563		GSE1456		GSE12276		GSE2109		GSE3494		GSE10248		GSE29271		GSE69031		GSE36771	
Number of patients	198		94		159		204		351		251		683		210		130		107	
Gene	R value	p-Value	R value	p-Value	R value	p-Value	R value	p-Value	R value	p-Value	R value	p-Value	R value	p-Value	R value	p-Value	R value	p-Value	R value	p-Value
TWIST1	0.177	0.013	0.012	0.909	0.281	3.28e-04	0.302	1.13e-05	0.441	3.61e-18	0.218	5.19e-04	0.393	1.07e-26	0.265	1.04e-04	0.334	1.01e-04	0.319	8.06e-04
SNAI1	0.132	0.064	0.109	0.297	0.252	1.38e-03	0.160	0.022	0.057	0.287	0.224	3.52e-04	0.234	6.24e-10	0.151	0.029	0.261	2.72e-03	0.234	0.015
SNAI2	0.181	0.011	0.250	0.015	0.280	3.46e-04	0.388	9.58e-09	0.375	3.94e-13	0.297	1.71e-06	0.352	2.35e-21	0.321	2.11e-06	0.257	3.14e-03	0.280	3.48e-03
ZEB1	0.058	0.419	0.086	0.408	0.237	2.61e-03	0.265	1.26e-04	0.511	8.78e-25	0.353	9.14e-09	0.421	1.05e-30	0.244	3.50e-04	0.331	1.21e-04	0.427	4.62e-06
ZEB2	0.042	0.556	0.103	0.323	0.290	2.12e-04	0.356	1.67e-07	0.468	1.59e-20	0.382	3.71e-10	0.467	3.31e-38	0.341	4.23e-07	0.364	2.08e-05	0.510	1.96e-08
CDH2	0.171	0.016	0.212	0.040	0.244	1.95e-03	0.136	0.052	0.098	0.068	0.100	0.114	0.126	9.44e-04	0.117	0.090	0.194	0.027	–0.066	0.496
VIM	0.445	5.00e-11	0.347	6.01e-04	0.386	5.07e-07	0.550	1.60e-17	0.487	2.83e-22	0.518	1.34e-18	0.525	1.02e-49	0.536	4.91e-17	0.387	5.47e-06	0.603	6.21e-12
MMP2	0.265	1.61e-04	0.097	0.355	0.147	0.064	0.244	4.43e-04	0.338	7.41e-11	0.279	7.02e-06	0.396	3.90e-27	0.208	2.49e-03	0.311	3.10e-04	0.466	4.18e-07
MMP9	0.163	0.022	0.261	0.011	0.003	0.971	0.374	3.56e-08	0.003	0.949	–0.019	0.760	0.076	0.047	0.359	8.59e-08	0.054	0.544	0.234	0.015
FN1	0.131	0.067	0.268	9.01e-03	0.117	0.141	0.202	3.72e-03	–0.048	0.368	0.192	2.24e-03	0.052	0.174	0.171	0.013	0.152	0.084	0.107	0.272

based on lymph node-negative status. To analyze correlation between *CD146* gene expression and expression of 11 selected mesenchymal profile genes, p-value of the Spearman correlation was considered significant below 0.0045 according to Bonferroni correction (0.05/11). In addition, using R2 database we analyzed gene expression data from 51 breast cancer cell lines (GSE 12777). All statistical analysis described in the article were performed using Statistica 12 (Statsoft Polska). All affymatrix gene expression array data were normalized using the MAS5 algorithm.

Results

Analysis of correlation between mesenchymal genes and *CD146* in breast cancer datasets

Initially, using the publicly available R2 database, we calculated Spearman rank correlation coefficient for *CD146* and 11 well known genes connected with mesenchymal profile including transcription factors coding genes (*SNAI1, SNAI2, ZEB1, ZEB2, TWIST1*), matrix metalloproteinases coding genes (*MMP2, MMP9*), fibronectin, *N*-cadherin and vimentin coding genes. In Table 1, the mean expression of all analyzed genes in 10 breast cancer datasets is shown. As determined in Table 1A, significant correlation was found between *CD146* and mesenchymal markers in all analyzed breast cancer datasets, but the number of correlated genes was different between the different datasets, i.e., in datasets with confirmed lymph node-negativity (only two correlated genes out of 11 tested) in comparison to other datasets (at least seven correlated genes out of 11, where lymph node-negativity was not taken into account during selection of patients material). Subsequently, we also analyzed the correlation between *CD146* gene expression and 11 mesenchymal profile genes in dataset composed of 51 breast cancer cell lines (Table 1B). As determined in Table 1C (left panel), most of the mesenchymal markers (eight out of 11) were correlated with *CD146* in analyzed cell lines dataset. It is noteworthy that *VIM* gene was the only gene significantly correlated with the *CD146* gene in all analyzed patient's date sets, whereas in cell lines, apparently, the highest correlation coefficient was observed for *VIM* and *CD146* gene (Table 1C, left panel). As breast cancer cell line dataset was composed of the cell lines with luminal and basal characteristics, which resemble, respectively, epithelial and mesenchymal gene expression profile [10], we performed additional analysis separately for basal and luminal breast cancer cell lines (Table 1C, middle panel and right panel). Interestingly, this analysis revealed that the *VIM* gene was the only one correlated positively with *CD146* gene in breast cancer cell lines with luminal characteristics, what indicates, in agreement with breast cancer patients data, that the relation between *CD146* and *VIM* gene is present regardless of the epithelial or mesenchymal gene expression profile.

Table 1B: Characteristics of 51 breast cancer cell lines used in the analysis.

Cell name	Subtype	Cell name	Subtype
bt483	luminal	bt20	basal
cama-1	luminal	bt-549	basal
efm19	luminal	cal-51	basal
evsa-t	luminal	cal85-1	basal
hcc1428	luminal	cal-120	basal
hcc1500	luminal	cal-148	basal
kpl1	luminal	du4475	basal
mcf7	luminal	hcc38	basal
mda-mb-175vii	luminal	hcc70	basal
mda-mb-415	luminal	hcc1143	basal
t47d	luminal	hcc1395	basal
zr75-1	luminal	hcc1806	basal
au565	luminal	hcc1937	basal
bt474	luminal	hdq-p1	basal
efm-192a	luminal	hs578t	basal
hcc202	luminal	mda-mb-134vi	basal
hcc1419	luminal	mda-mb-231	basal
hcc2218	luminal	mda-mb-435s	basal
jimt-1	luminal	mda-mb-436	basal
kpl-4	luminal	mda-mb-468	basal
mda-mb-361	luminal	mx1	basal
mda-mb-453	luminal	sw527	basal
mfm-223	luminal	hcc1569	basal
skbr3	luminal	hcc1954	basal
uacc-812	luminal		
uacc-893	luminal		
zr75-30	luminal		

Spearman's rank correlation coefficients matrix analysis in breast cancer datasets

Initially, the Spearman's rank correlation coefficients were calculated between the expression level of the specified genes (coding mesenchymal markers) and the level of *CD146* gene expression in 10 independent breast cancer datasets. Spearman's rank coefficient enables correlations finding of any monotonic dependence, without assuming linearity. The calculations were performed for each dataset separately. Then each value of the correlation coefficient was modified according to the following formula:

$$r = \{r = r \text{ for } p < 0.0045\, r = 0 \text{ for } p \geq 0.0045\}$$

taking zero as the value of correlation coefficients for

Table 1C: Spearman's rank coefficients between *CD146* and 11 mesenchymal markers genes in 51 breast cancers cell lines transcriptomic datasets.

GEN	Number of breast cancer cell lines	R value	p-Value	Number of luminal breast cancer cell lines	R value	p-Value	Number of basal breast cancer cell lines	Spearman r	p-Value (two-tailed)
TWIST1	51	0.286	0.042	27	–0.0812	0.6872	24	0.4462	0.0289
SNAI1	51	0.156	0.275	27	–0.4137	0.032	24	0.08263	0.7011
SNAI2	51	0.683	3.28e-08	27	–0.1218	0.545	24	0.6214	0.0012
ZEB1	51	0.477	4.08e-04	27	0.1121	0.5776	24	0.3235	0.123
ZEB2	51	0.446	1.04e-03	27	–0.1656	0.4092	24	0.4519	0.0266
CDH2	51	0.535	5.19e-05	27	–0.3413	0.0814	24	0.6825	0.0002
VIM	51	0.772	3.32e-11	27	0.453	0.0177	24	0.6023	0.0018
MMP2	51	0.472	4.74e-04	27	–0.1609	0.4227	24	0.4837	0.0166
MMP9	51	0.186	0.192	27	–0.0757	0.7074	24	0.04566	0.8322
TWIST2	51	0.398	3.79e-03	27	–0.1176	0.5592	24	0.139	0.5172
FN1	51	0.569	1.30e-05	27	–0.1142	0.5707	24	0.7184	0.0001

Left panel: Analysis of 51 breast cancer cell lines composed of 27 luminal and 24 basal ones, Middle panel: Analysis of 27 luminal breast cancer cell lines, Right panel: Analysis of 24 basal breast cancer cell lines. For significant positive correlations the R value and p-value are marked in gray.

Table 2: Spearman's rank correlation coefficients modified according to Bonferroni correction between *CD146* and 11 mesenchymal markers genes in 10 breast cancer transcriptomic patients' datasets.

GEO ID	GSE7390	GSE46563	GSE1456	GSE12276	GSE2109	GSE3494	GSE10248	GSE29271	GSE69031	GSE36771
Number of patients	198	94	159	204	351	251	683	210	130	107
Gene	R value	R value	R value	R value	R value	R value	R value	R value	R value	R value
TWIST1	0	0	0.281	0.302	0.441	0.218	0.393	0.265	0.334	0.319
SNAI1	0	0	0.252	0	0	0.224	0.234	0	0.261	0.234
SNAI2	0	0	0.28	0.388	0.375	0.297	0.352	0.321	0.257	0.280
ZEB1	0	0	0.237	0.265	0.511	0.353	0.421	0.244	0.331	0.427
ZEB2	0	0	0.290	0.356	0.468	0.382	0.467	0.341	0.364	0.510
CDH2	0	0	0.244	0	0	0	0.126	0	0	0
VIM	0.445	0.347	0.386	0.550	0.487	0.518	0.525	0.536	0.387	0.603
MMP2	0.265	0	0	0.244	0.338	0.279	0.396	0.208	0.311	0.466
MMP9	0	0	0	0.374	0	0	0	0.359	0	0
FN1	0	0.268	0	0.202	0	0.192	0	0	0	0

which the significance level p was greater than or equal to 0.0045. The modified correlation coefficients of individual genes with the *CD146* gene for selected datasets are presented in Table 2. Next, cluster analysis was performed using the square of Euclidean distance.

$$d(x,y) = \sum ni = 0 (x_i - y_i)^2$$

Subsequently, single linkage method was chosen as a hierarchical method to estimate the distance between clusters. In this method the distance between two clusters is defined by the distance between the closest objects (nearest neighbors) belonging to different clusters. On this basis, the distance matrix was calculated (Table 3A). The distance matrix is visualized on the dendrogram presented in Figure 1 and interpreted based on coefficients mean, coefficients of variation and the values of distance (Table 3A and Table 3B). Interestingly, it has been observed that the set of correlation coefficients for the *VIMENTIN* gene with *CD146* gene is the most different from the others. It has the highest mean correlation coefficient with the *CD146* gene, the lowest coefficient of variation (Table 3B) and the largest distance (Table 3A) from the others what may indicate that *VIM* gene is regulated directly by the same mechanism as the *CD146* gene, or the *CD146* and *VIM* gene directly regulate one another during the induction of the mesenchymal profile. The remaining genes have a lower mean correlation coefficient and a higher coefficient of variation (Table 3B). Moreover, they form completely separate branches on the dendrogram, as determined in Figure 1 in relation to the *VIMENTIN* gene.

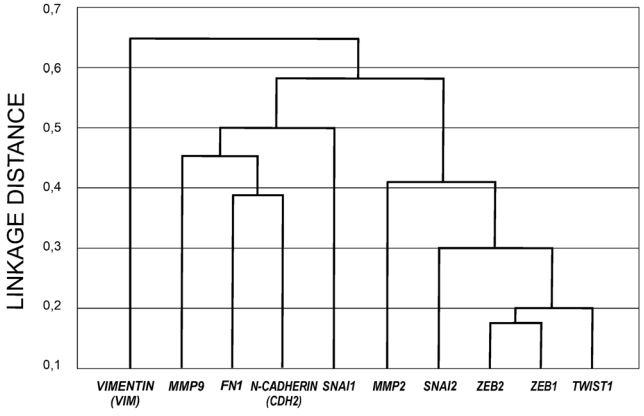


Figure 1: The tree diagram of correlation coefficients between 11 mesenchymal profile genes and *CD146* gene in 10 independent breast cancer patients transcriptomic datasets.

Discussion

Several independent studies confirmed the existence of correlation between *CD146* and highly motile mesenchymal profile of cancer cells [1]. In our current analysis, we confirmed this finding analyzing the correlation between mesenchymal profile genes (the hallmark of EMT) and the expression of *CD146* in 10 transcriptomic breast cancer patients datasets. Nevertheless, still little is known about the regulation of CD146 expression in breast cancer and it is not clear if aberrant *CD146* is always required in EMT process. Importantly, *in vitro* studies in breast cancer cells revealed that ectopic expression of *CD146* in epithelial line is sufficient to induces the mesenchymal profile, nevertheless we cannot exclude that *in vivo CD146* is triggered by the upstream signaling inducing EMT. One note, high *CD146* gene expression is observed especially in triple negative tumors (TNBC) [3], what may indicate the role of estrogen, progesterone or Her2 signaling in CD146-induced EMT. Indeed, in analyzed breast cancer datasets the significant negative correlation has

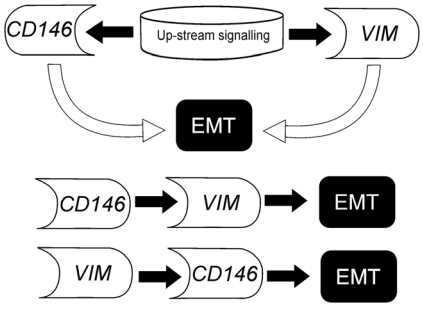
Table 3A: The matrix of linkage distance.

	TWIST1	*SNAI1*	*SNAI2*	*ZEB1*	*ZEB2*	*CDH2*	*VIM*	*MMP2*	*MMP9*	*FN1*
TWIST1	0.00	0.58	0.30	0.20	0.28	0.86	0.81	0.44	0.88	0.89
SNAI1	0.58	0.00	0.64	0.66	0.74	0.50	1.16	0.63	0.74	0.61
SNAI2	0.30	0.64	0.00	0.37	0.40	0.83	0.71	0.53	0.76	0.80
ZEB1	0.20	0.66	0.37	0.00	0.18	0.97	0.76	0.47	1.0	0.97
ZEB2	0.28	0.74	0.40	0.18	0.00	1.08	0.66	0.47	1.06	1.08
CDH2	0.86	0.50	0.83	0.97	1.08	0.00	1.42	0.93	0.59	0.40
VIM	0.81	1.16	0.71	0.76	0.66	1.42	0.00	0.79	1.31	1.38
MMP2	0.44	0.63	0.53	0.41	0.47	0.93	0.79	0.00	0.92	0.88
MMP9	0.88	0.74	0.76	1.00	1.06	0.59	1.31	0.925	0.00	0.44
FN1	0.89	0.61	0.80	0.97	1.08	0.40	1.38	0.88	0.44	0.00

Table 3B: Mean and standard deviation of Spearman's rank correlation coefficients and coefficients of variation for 11 mesenchymal markers genes correlated with *CD146* gene in 10 independent transcriptomic breast cancer patients' datasets.

Gene	Mean	Standard deviation	Coefficient of variation
TWIST1	0.25	0.15	0.58
SNAI1	0.14	0.12	0.89
SNAI2	0.28	0.11	0.39
ZEB1	0.28	0.17	0.61
ZEB2	0.32	0.18	0.57
N-CADHERIN (CDH2)	0.069	0.10	1.68
VIMENTIN (VIM)	**0.48**	**0.08**	**0.17**
MMP2	0.25	0.15	0.61
MMP9	0.1	0.16	1.64
FN1	0.07	0.11	1.64

been found (data bot shown) for estrogen receptor (five out of 10 breast cancer patients dataset) and progesterone receptor (four out of 10 breast cancer patients datasets). Moreover, the literature data revealed the decrease in estrogen receptor expression in cells undergoing CD146-induced EMT [11]. In contrast, we did not find any significant correlation between *CD146* and Her2 coding gene (*ERBB2*) in 10 analyzed datasets (data not shown). Most probably the fact that *ERBB2* is often amplified, leading to induction of mesenchymal profile [12], and at the same lost in the group of TNBC tumors (characterized by aggressive mesenchymal expression profile and high *CD146* expression) makes the potential relation between these two genes difficult to be analyzed based on gene expression profile. Another noteworthy scenario is that described in melanoma cells, where expression of the *CD146* gene alone activates the endogenous PI3K/AKT pathway, which in a positive feedback induces *CD146* expression. The PI3K/Akt signaling pathway interacts with other pathways, either directly or indirectly inducing EMT, resulting in increased aggressiveness, including proliferation, resistance to apoptosis, invasiveness, metastasis and resistance to chemotherapy. It is therefore possible that the abnormal overexpression of the *CD146* gene itself will induce signaling leading to an epithelial-mesenchymal transition via activation of the PI3K/Akt pathway [6]. What is more, the fact that activation of PI3/Akt promotes *CD146* expression in a feedback mechanism, may explain why high *CD146* expression maybe crucial to maintain the mesenchymal profile in cancer cells. In other studies, *CD146* has been shown to mediate E-cadherin-to N-cadherin switch during TGF-beta-induced EMT transition. As the authors of the study showed, the *CD146* expression was positively correlated with the STAT3/Twist activation and the ERK pathway. It has been shown that activation of CD146/STAT3/Twist signaling inhibits E-cadherin, while CD146/ERK enhances N-cadherin expression [13]. Regardless, the regulation scenario in breast cancer our correlation coefficients analysis revealed the closest relationship between *CD146* expression and *VIMENTIN* expression in breast cancer patients datasets, which implies that a potential mechanism of *CD146* regulation may be directly related to the regulation of the *VIM* gene. What is more, the *VIM* gene was the only one correlated with *CD146* in all patients datasets, regardless the lymph node-negativity status and it was also the only gene significantly correlated with *CD146* in luminal breast cancer cell lines, which resemble the tumors with epithelial gene expression profile. Altogether, it indicates that the correlation between *CD146* and *VIM* gene expression is present in regardless to gene expression profile, epithelial or mesenchymal. In addition, of particular importance is the fact that both, the expression of vimentin itself and the expression of *CD146* itself may lead to the induction of the mesenchymal profile in breast cancer cells [3, 14]. The question then remains whether CD146 is necessary for induction of the mesenchymal profile by vimentin and *vice versa*. In addition, we should take into account the fact that both markers correlate closely with the aggressiveness of breast cancer and understanding the mechanisms of their potential co-regulation may significantly contribute to the development of new therapies targeting mesenchymal cancer cells responsible for metastasis and the same progression of the disease. Based on the results of the analysis, we propose the model of potential CD146/VIMENTIN coregulation, which should be tested *in vitro* (Scheme 1) in breast cancer cells. This model is based on three assumptions, the first that upstream signaling triggers the expression of *CD146* and *VIMENTIN* which independently induce the mesenchymal profile or that *CD146* triggers the expression of *VIM* gene (or *vice versa*) leading to the induction of the mesenchymal profile. Of particular importance is the fact that vimentin silencing leads to downregulation of mesenchymal profile genes [14] and the same was observed for CD146 [3]. The crucial question remains if vimentin-dependent induction of EMT



Scheme 1: The hypothetical model presenting the potential relation between *VIM* and *CD146* gene co-expression in breast cancer cells. VIM, vimentin; EMT, epithelial to mesenchymal transition.

requires CD146 and if CD146-dependent induction of EMT requires vimentin. Undoubtedly, testing of these research hypotheses *in vitro* may significantly expand the knowledge about EMT in breast cancer cells and contribute to the development of new therapeutic strategies targeting highly metastatic breast cancer cells.

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