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CLINICAL STUDY

Investigation of serum E-Cadherin, VEGF121, Survivin, Tenascin C and Tetraspanin 8 levels in patients with glioblastoma

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ABSTRACT

OBJECTIVE: The aim of this study is to determine biomarkers, which may be used in order to understand the pathophysiology, the diagnosis, progression surveillance/monitoring, and treatment efficacy of high graded glial tumors.

BACKGROUND: Radiological imaging in the diagnosis and relapse surveillance of glial tumors is sometimes insufficient. There is need for additional methods of diagnosis and surveillance in order to rule out contradictory circumstances.

METHOD: Using enzyme like immune sorbent assay method, E-Cadherin, Tenascin C, Tetraspanin 8, Survivin and VEGF121 levels were investigated in serum and tumor tissues of 28 patients diagnosed with pathological glioblastoma, and in the serum of 26 healthy individuals. Correlation between tumor tissue values and Ki67 percentage, and P53 mutation, and difference between unhealthy and healthy serum levels were sought.

RESULTS: It was found out that E-Cadherin and VEGF 121 levels in the unhealthy serum were high in comparison to the control group ($p < 0.05$). In the patient group, there was no correlation determined between tissue and serum levels of all biomarkers and mutation percentages of Ki67 and p53 ($p > 0.05$).

CONCLUSION: EC and VEGF121 are biomarkers, which have the potential to be used in the diagnosis, recurrence and treatment follow-up in high graded glial tumors *(Tab. 2, Fig. 1, Ref. 37)*. Text in PDF *www.elis.sk*

KEY WORDS: E-Cadherin, VEGF, Survivin, Tenascin-C, Tetraspanin, glioblastoma.

Abbreviations: glioblastoma (GB), vascular endothelial growth factor (VEGF), E-Cadherin (EC), Tenascin C (TN-C), Tetraspanin-8 (Tspan8), enzyme-like immune sorbent assay (ELISA)

Introduction

Glioblastoma (GB) is the most common, and also the most malignant tumor of the central nervous system in adults (1). Despite the advances in microsurgery approaches and oncological treatments, these tumors have a poor prognosis with an average of 14 months of life expectancy (2). In these tumors, which are very difficult to manage, the biomarkers in their content gain importance for the understanding of the tumor biology and behavioral differences, and for the use of them in an objective classification, prognosis determination, personalized therapeutic decision making, diagnosis and follow-up of recurrence (3).

Vascular endothelial growth factor (VEGF) is known as the most important mediator in the neovascularization in GBs, and their serum level increases (4, 5). Today, six splice isoforms of VEGF are identified, and $VEGF_{xxx}$ family consisting of mature peptides has been formed (6). VEGF121 is a member of this family and its pro-angiogenic potential is high (7).

Cadherin family members are transmembrane glycoproteins who are responsible for calcium-dependent cell adhesion. E-Cadherin (EC) is the member of this family, which tends to be found in zonula adherence regions of epithelial cells (8). During tumor progression, its expression decreases frequently (9). Loss or reduction of EC expression is associated with aggressiveness and dedifferentiation of many carcinomas (10).

Surivivin has a role in both apoptosis inhibition and cellular division (11). While having minimal expression in healthy tissues, overexpression is detected in many cancer types, in which the cell cycle is impaired (12). The overexpression of Survivin in cancerous cells has been associated with tumor progression, poor prognosis, and therapeutic resistance in these cells (13).

Tenascin C (TN-C) is an adhesion modulating glycoprotein, found in the extracellular matrix consisting of specific polypep-

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tides (14). It is overexpressed in the embryological period during organogenesis, where cell proliferation, migration, epithelial mesenchymal transition occurs (15). During the development of the central nervous system, TN-C plays a role in both the regulation of both oligodendrocyte blast cells and the reproduction of astrocytes (16).

Tetraspanin-8 (Tspan8) is one of the 4 cell surface proteins called Tetraspanins and has very important cellular functions such as cell migration, adhesion, invasion and proliferation (17). Tspan 8 overexpression is observed in many cancer types and is associated with poor prognosis (18).

GB patients are lost due to tumor cell infiltration and local invasion. Unfortunately, conventional chemotherapy and radiotherapy at post-surgical period cannot resist this invasion. New therapeutic targets, which can be added to the follow-up and treatment protocol should be investigated and revealed (19). In this study, we examined VEGF121, EC, Survivin, TN-C and Tspan 8 levels in the serum of healthy individuals and in the serum and tumor tissue of patients with high-graded masses, and investigated the correlation between Ki67 and P53 rates, which are associated with the tumor aggressivity, using the enzyme-like immunosorbent assay (ELISA) method.

Material and method

Patient samples

Twenty-eight patients above the age of 18 with a diagnosis of GB and 26 volunteers without any cancer were included in the study. Tumor tissues and preoperative blood samples were obtained from patients who were operated due to high graded glial tumor between June 2019 and August 2021, and blood samples of volunteer outpatients coming to the clinic for various reasons were also obtained for this research. GB cases were confirmed by magnetic resonance imaging (MRI) and histopathology according to the 2021World Health Organization classification (20). Those with recurrent glial masses, those who had intracranial operation, those with central nervous system disease, those with a different history of cancer, and those who underwent chemotherapy or radiotherapy were excluded from the study. The approval for this prospective study was granted by the Namik Kemal University Ethics Committee. (Date: 25.04.2019 number: 2019.58.04.05) The research protocol was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all study subjects after the explanation of the nature and possible consequences of the study. Blood samples before anesthesia induction, were obtained from patients who were planned to be operated for their tumors. Tumor tissues were tried to be taken from the midpoint of the tumor, and from the contrast- enhancing areas of the tumor, and each tumor tissue sample was divided into two for the pathological verification and was kept in dry tubes as well as in tubes containing formol. When more than one cc of tumor samples was collected, tissues in dry tubes and preoperative blood samples were kept in the cooler at –80 °C. Peripheral blood samples obtained from the patients included in this study in a red capped gel tube were centrifuged for fifteen minutes at 1000xg in order to obtain serum. Serum samples which were obtained, were separated into microcentrifuge tubes. The separated serum and tissue samples were kept in a deep-freezer (–80 °C) until the day of the analysis. Immunohistochemically, Ki67 and P53 antibodies were applied to the pathology preparations of patients. Both antibodies revealed nuclear staining in tumor cells, and the percentages of tumor cells, which were stained were determined via a microscope and were counted. The association between protein amounts of the tumor tissue and Ki67 index and P53 gene mutation was investigated, and the difference between biomarker levels of patient serums and control groups were also calculated.

Biomarker analysis

Collected serum samples were brought to room temperature on the day of analysis. The tissue samples, which were to be analyzed, were cut into pieces of the same size and were washed via phosphate buffer (0.01M pH: 7.4). Tissue samples, which were quickly dried, were homogenized in a volume of buffer proportional to their weight after weighing (buffer was used 9 times the tissue weight). Homogenates were transferred into tubes, and they were centrifuged for 20 minutes in 2500 x g. The supernatant part within the tubes after centrifugation was transferred into microcentrifuge tubes and was frozen. The supernatant part was used in the analysis. Serum and tissue sample levels of the patients who were included in the study were measured via ELISA method.

Statistical analysis

Power analysis was performed with the effect size (d) = 0.8, $\alpha = 0.05$, power (p) = 0.8, N2/N1 = 1 using the G-power 3.0.10 program, and it was calculated that the groups would consist of at least 26 people. Therefore, 28 patients and 26 controls were taken to account for a total sample of 54 individuals.

SPSS package program was used for the statistical analysis. The normality test of the data was checked. Independent sample t test (or Mann–Whitney u test) was used in the comparison of the two independent groups. The results were given as mean ± SD. The correlation of the continuous variables was tested using Pearson (or Spearman) correlation analysis.

Results

While the average age of the patient group was 58.57 ± 10.79 years, it was 57.95 ± 13.26 years in the control group. Both groups were similar in terms of age (p = 0.859). While there were 18 female and 10 male patients in the patient group, the control group consisted of 13 female and 13 male patients, and there was no difference in terms of gender between the two groups (p = 0.488). Characteristic features of the patient and control groups as well as their Tenascin C, Tetraspanin-8, E-Cadherin, VEGF 121 and Survivin serum levels are given in Table 1.

It was observed that blood levels of all biomarkers in the patient group were higher in comparison to the control group. While E-Cadherin levels in the patient group were found to be 127.38 ± 44.44 ng/ml, it was that there was a significant difference (p = 0.019) with the control group, the serum level of which was 95.38

Tab. 1. Patient and control group characteristics and serum biomarker levels and differences.

	Group		Mean ± sd/n	p
Age	patient		58.57±10.79	0.859 [t]
	control		57.95±13.26	
Gender	patient	female	18(%64.3)	0.488 [x2]
		male	10(%35.7)	
	control	female	13(%50)	
		male	13(%50)	
Tenascin C	patient		18.39±3.94(ng/ml)	0.055 [t]
	control		15.3 ±6.85(ng/ml)	
Tetraspanin-8	patient		4.401±1.063(ng/ml)	0.094 [t]
	control		3.797±1.385(ng/ml)	
E-Cadherin	patient		127.38±44.44(ng/ml)	0.019 [t]
	control		95.38±45.51(ng/ml)	
VEGF 121	patient		224.78±68.9(ng/ml)	0.000 [t]
	control		130.12±50.23(ng/ml)	
Survivin	patient		95.46±73.92(ng/ml)	0.051 [t]
	control		61.07±23.12(ng/ml)	

*Values with statistical significance are marked in bold. sd: standart deviation, ng/ml: nanograms per milliliter, t t-test / χ^2 Chi-squrae test

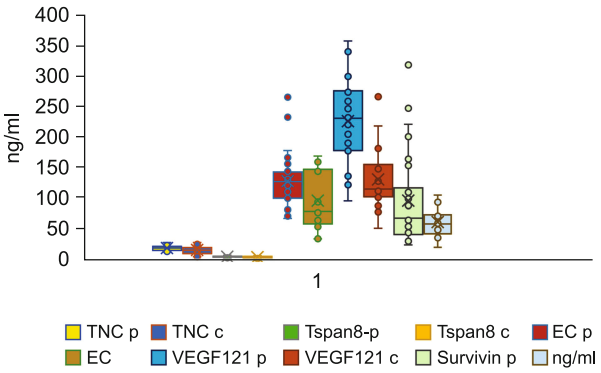

Fig. 1. Distribution of biomarker levels in patient (p) and control (c) groups.

± 45.51 ng/ml. It was determined that the biggest difference was in serum VEGF 121 levels (p = 0.000) While VEGF 121 level in the patient group was 224.78±68.9 ng/ml, it was determined as 130.12 ± 50.23 ng/ml in the control group. When serum Tenascin C, Tetraspanin-8, and Survivin levels of both patient and control groups were analyzed, there was no difference found between the two groups (p = 0.055, p = 0.094, p = 0.051, respectively) (Tab. 1). The intergroup distribution of all biomarkers is shown in Figure 1.

There was no correlation of tissue and serum levels of all biomarkers in the patient group and Ki67 percentages and p53 mutation values. The correlation between Ki67 percentage, p53 mutation, and biomarkers are shown in Table 2.

Discussion

Gliomas are the most common and most malignant brain tumors arising from neural epithelial cells and have a poor prognosis due to their rapid recurrence. The treatment method, which prolongs the prognosis, is still the surgical total removal. In order to cope with this group of tumors, it is necessary to find new diagnostic, follow-up, and treatment methods. In our study, we revealed that E-Cadherin and VEGF 121 levels in patients with high graded glial tumors elevated significantly, while there was no significant change in Tenascin C, Tetraspanin-8, or Survivin levels.

VEGF is also an angiogenesis inhibitor family, expressed in the endothelium (21). Its expression as an angiogenesis inhibitor brings about the possibility of it becoming an independent prognostic factor. Mentlein et al stated that there was significantly high level of expression in the $VEGF_{121}$ and $VEGF_{165}$ splice variants in GBs (22). Martini et al (23), on the other hand, mentioned that $VEGF_{121}$ levels decreased in cases, where bevacizumab, an anti VEGF monoclonal antibody, was used in the treatment of recurrent gliomas, and that it is associated with good prognosis. Moreover, it was also reported that in cases with recurrent GBM, $VEGF_{121}$ protein plasma level was threefold higher than the levels of healthy volunteers, and that the mRNA isoform in their tumor tissue had a higher expression in patients with high level of plasma $VEGF_{121}$ (23). In children using bevacizumab in the treatment of gliomatosis cerebri, the plasma $VEGF_{121}$ level decreases (24). This decrease can be correlated with the decrease in tumor tissue and give an idea about the efficacy of the treatment. In our study, $VEGF_{121}$ plasma protein level was found to be significantly higher in GBM patients when compared to healthy volunteers, but this elevation was not correlated with Ki67 positivity and p53 mutation. Our study as well as other studies have revealed that $VEGF_{121}$ plasma level can be used in the understanding of the chemotherapy efficacy or the follow-up of recurrence.

In the studies carried out by Yang et al (25), in which they investigated the TGF- β1 and EC expressions, they found out that while TGF- β1 expression in glioma tissue was high, EC expression was found to be low, and that there was a negative correlation between TGF- β1 and EC expressions. Rodriguez et al (26), have

Tab. 2. Correlation of Ki67 index, P53 mutation with serum and tissue levels of biomarkers.

		Tenascin C (Tumor)	Tenascin C (Serum)	Tetraspanin-8 (Tumor)	Tetraspanin-8 (Serum)	E-Cadherin (Tumor)	E-Cadherin (Serum)	VEGF 121 (Tumor)	VEGF 121 (Serum)	Survivin (Tumor)	Survivin (Serum)
Ki67	R	–0.11	–0.058	0.103	–0.253	0.092	–0.332	0.066	–0.118	0.038	0.021
	p	0.547	0.768	0.600	0.193	0.641	0.085	0.739	0.549	0.849	0.915
P53	r	0.184	–0.058	0.271	0.174	0.005	–0.109	0.282	0.202	–0.093	–0.18
	p	0.350	0.769	0.164	0.377	0.979	0.582	0.146	0.302	0.638	0.359

r = Pearson correlation

stated that there was EC staining in 82 % of the GBs. On the other hand, Noh et al. reported that EC expression in GBs was 8.7 %, and N-Cadherin expression was 88 % (27). There is a limited number of publications, which similarly to this study, reveal decrease or increase in EC expression. A group of researchers also studied the correlation of EC and prognosis and have stated different point of views.

However, in our study, EC levels in patient serum were found to be significantly higher in comparison to the control group. This elevation means that the EC expression increases. We also found out that EC expression did not reveal any correlation with Ki67 and p53 gene mutations, which are related to tumor aggressivity.

While Survivin had minimal expression in normal and healthy tissues, it was found out that there was overexpression in many cancer types, in which the cell cycle was impaired (12). The overexpression of Survivin in cancerous cells is correlated with tumor progression, poor prognosis, and therapeutic resistance in these cells (13). There are limited publications reporting increased levels of Surviving in malignant brain tumors (28). However, in our study, Survivin levels in the patient group were found to be higher than in the control group, yet the difference was statistically insignificant.

It is known that TN-C expression increased in many cancer types, but mostly in malignant glial tumors (29). There are studies, which reveal that this may be a potential prognostic factor in gliomas (30, 31). While TN-C can be found in perivascular and intercellular areas in gliomas, it also can be found in small amounts within cells, and it was also reported that its amount increases as the grade increases in the cerebrospinal fluid (32, 33). Considering all these results, while our expectation was to see a rise in the serum TN-C levels, in our study, for some reason, there was no significant change in the levels when compared to the TN-C levels in the control group. Even if TN-C tumor tissue concentration is high, and even when there is a transition into the cerebrospinal fluid, it is interesting that it cannot be detected in the systemic circulation, which may be the subject of another research.

In the studies related to Tspan8, it is known that it is secreted in small amounts in normal tissues, while it is expressed in some cancer types such as colon, liver, prostate, ovarian and cervical cancers (34). Its high expression indicates poor prognosis particularly in colon carcinomas (35). There is little information about the relationship between high graded glial masses and Tspan8 in scientific literature. In their study on glioma cell lines, Pan et al. reported that Tspan8 might contribute the tumor pathology in terms of proliferation, migration, and temozolomide resistance, and that it may be a therapeutic target in anti-glioma therapy (36). In our study, however, Tspan8 serum levels were not found to be high, and there was no correlation determined between this serum and Ki67 and p53 mutation.

Cell lines were used in many of the studies in scientific literature, in which similarly biomarker levels were measured. Studies investigating patient and serum levels are quite few. There is a dynamic classification system in high graded glial tumors, and it is renewed frequently. The reason for this search is the observation of different clinical results in patients with the same pathological grades with different glial tumors. At this point, it is essential that various mediators be revealed for the understanding of the biology of a tumor. The relationship between the biomarkers, which we have investigated, and the progression of the disease should be kept in mind in terms of recurrence, or for the follow up of the response to treatment.

Conclusion

In our study, EC and VEGF121 levels in patient serums of high graded glial masses were found to be high. There was no correlation found between any of the studied biomarkers and Ki67 and p53 gene mutations EC and VEGF are biomarkers, which have the potential to be used in the diagnosis, recurrence, and treatment follow-up of high graded glial tumors.

References

1. Ostrom QT, Gittleman H, Fulop J, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2008– 2012. Neuro Oncol 2015; 17 (Suppl 4): iv1–iv62.

2. Schmidinger M, Linzmayer L, Becherer A, et al. Psychometric-and quality-of-life assessment in longterm glioblastoma survivors. J Neurooncol 2003; 63 (1): 55–61.

3. Linhares P, Carvalho B, Vaz R, Costa BM. Glioblastoma: Is There Any Blood Biomarker with True Clinical Relevance? Int J Mol Sci 2020; 21 (16): 5809.

4. Franco-Hernandez C, Martinez-Glez V, Rey JA. Biology molecular of glioblastomas. Neurocirugia (Astur) 2007 oct; 18 (5): 373–382.

5. Reynes G, Vila V, Martin M et al. Circulating markers of angiogenesis, inflammation, and coagulation in patients with glioblastoma. J Neurooncol 2011; 102 (1): 35–41.

6. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. Mol Endocrinol 1991; 5 (12): 1806–1814.

7. Keyt BA, Berleau LT, Nguyen HV et al. The carboxyl-terminal domain (111–165) of vascular endothelial growth factor is critical for its mitogenic potency. J Biol Chem 1996; 271 (13): 7788–7795.

8. Lewis JE, Jensen PJ, Johnson KR, Wheelock MJ. E-Cadherin mediates adherens junction organization through protein kinase C. J Cell Sci 1994; 107 (Pt12): 3615–3621.

9. Behrens J, Lowrick O, Klein-Hitpass L, Birchmeier W. The E-Cadherin promoter: final analysis of a G: C-rich region and an epithe- lial cell-specific palindromic regulatory element. Proc Natl Acad Sci USA 1991; 88 (24): 11495–11499.

10. Birchmeier W, Beherens J. Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta1994; 1198 (1): 11–26.

11. Mita AC, Mita MM, Nawrocki ST, Giles FJ. Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. Clin Cancer Res 2008; 14 (16): 5000–5005.

12. Ryan BM, O'Donovan N, Duffy MJ. Survivin: a new target for anticancer therapy. Cancer Treat Rev 2009; 35 (7): 553–562.

13. Li F, Ambrosini G, Chu EY et al. Control of apoptosis and mitotic spindle checkpoint by Survivin. Nature 1998; 396 (6711): 580–584.

14. Orend G, Chiquet-Ehrismann R. Tenascin-C induced signaling in cancer. Cancer Lett 2006; 244 (2): 143–163.

15. Midwood KS, Hussenet T, Langlois B, Orend G. Advances in Tenascin-C biology. Cell Mol Life Sci 2011; 68 (19): 3175–3199

16. Wiese S, Karus M, Faissner A. Astrocytes as a source for extracellular matrix molecules and cytokines. Front Pharmacol 2012; 3: 120.

17. Charrin S, Naour F le, Silvie O, Milhiet P E, Boucheix C, Rubinstein E. Lateral organization of membrane proteins: Tetraspanins spin their web. Biochem J 2009: 420 (2): 133–154.

18. Sala-Valdes M S, Ailane N, Greco C, Rubinstein E, Boucheix C. Targeting Tetraspanins in cancer. Expert Opin Ther Targets 2012; 16 (10): 985–997.

19. Westphal M, Lamszus K. The neurobiology of gliomas: From cell biology to the development of therapeutic approaches. Nat Rev Neurosci 2011; 12 (9): 495–508.

20. Louis DN, Perry A, Wesseling P et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol 2021; 23 (8): 1231–1251.

21. Kerbel RS. Vasohibin: the feedback on a new inhibitor of angiogenesis. J Clin Invest 2004; 114 (7): 884–886.

22. Mentlein R, Forstreuter F, Mehdorn HM, Held-Feindt J. Functional significance of vascular endothelial growth factor receptor expression on human glioma cells. J Neurooncol 2004; 67 (1–2): 9–18.

23. Martini M, Pascalis I, D'Alessandris QG et al. VEGF-121 plasma level as biomarker for response to anti-angiogenetic therapy in recurrent glioblastoma. BMC Cancer 2018; 18 (1): 553.

24. Ninomiya H, Ozeki M, Matsuzawa Y et al. A pediatric case of anaplastic astrocytoma with a gliomatosis cerebri; the growth pattern and changes in serum VEGF-121 levels after bevacizumab treatment . J Clin Neurosci 2020; 81: 431–433.

25. Yang L, Liu M, Deng C, Gu Z, Gao Y. Expression of transforming growth factor-β1 (TGF-β1) and E-Cadherin in glioma. Tumor Biol 2012; 33 (5): 1477–1484.

26. Rodriguez M, Aladowicz E, Lanfrancone L, Goding CR . Tbx3 represses E-Cadherin expression and enhances melanoma invasiveness. Cancer Res 2008; 68 (19): 7872–7881.

27. Noh MG, Oh SJ, Ahn EJ, et al. Prognostic significance of E-Cadherin and N-Cadherin expression in Gliomas. BMC Cancer 2017; 17 (1): 583.

28. Kafadar D, Yaylim I, Kafadar AM et al. Investigation of Survivin Gene Polymorphism and Serum Survivin Levels in Patients with Brain Tumors. Anticancer Res 2018; 38 (10): 5991–5998.

29. Orend G, Chiquet-Ehrismann R. Tenascin-c induced signaling in cancer. Cancer Lett 2006; 244 (2): 143–163.

30. Leins A, Riva P, Lindstedt R, Davidoff MS, Mehraein P, Weis S. Expression of Tenascin-c in various human brain tumors and its relevance for survival in patients with astrocytoma. Cancer 2003; 98 (11): 2430–2439.

31. Nie S, Gurrea M, Zhu J et al. Tenascin-c: A novel candidate marker for cancer stem cells in glioblastoma identified by tissue microarrays. J Proteome Res 2015; 14 (2): 814–822.

32. Kim CH, Bak KH, Kim YS et al. Expression of Tenascin-c in astrocytic tumors: its relevance to proliferation and angiogenesis. Surg Neurol 2000; 54 (3): 235–240.

33. Yoshida J, Wakabayashi T, Okamoto S et al. Tenascin in cerebrospinal fluid is a useful biomarker for the diagnosis of brain tumour. J neurology neurosurgery Psychiatry 1994; 57 (10): 1212–1215.

34. Ailane N, Greco C, Zhu Y, et al. Effect of an anti-human Co-029/tspan8 mouse monoclonal antibody on tumor growth in a nude mouse model. Front Physiol 2014; 5: 364.

35. Greco C, Bralet MP, Ailane N, et al. E-Cadherin/p120-catenin and Tetraspanin Co-029 cooperate for cell motility control in human colon carcinoma. Cancer Res 2010; 70 (19): 7674–7683.

36. Pan S-J, Wu Y-B, Cai S, et al. Over-expression of Tetraspanin 8 in malignant glioma regulates tumor cell progression. Biochem Biophys Res Commun 2015; 458 (3): 476–482.

37. Larue L, Ohsugi M, Hirchenhain J, Kemler R. E-Cadherin null mutant embryos fail to form a trophectoderm epithelium. Proc Natl Acad Sci USA 1994; 91 (17): 8263–8267.

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