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Nectin-4 In Triple Negative Breast Cancers - A Comprehensive Overview

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ABSTRACT— Nectin-4 is a novel, highly useful biomarker for many solid cancers including breast cancer, recently gaining focus of cancer researchers all over the globe due to its potential of being a target for molecular therapy for difficult to treat cancers such as triple negative breast cancer (TNBC). Recent studies have shown that increased Nectin-4 activity is strongly connected to tumour incidence and progression in a variety of cancer types, although it is still unclear how Nectin-4 protein affects the initiation and progression of these cancers. In this review article, we have highlighted the mechanisms underlying, biological activities, and recent developments pertaining to the Nectin-4 protein expression pattern in diverse cancer types, mainly emphasizing on its role in the carcinogeneisis of TNBC. Also, we have summarised various methods of detection and scoring used by various researchers.

KEYWORDS: Molecular, carcinogenesis, genetics, oncoprotien, target, chemotherapy

1. INTRODUCTION

The increasing importance of targeting drugs in the treatment of several cancers such as breast, colon, lung, malignant melanoma, lymphoma and many others has lead to the importance understanding the molecular pathology of various cancers [1]. Five transmembrane glycoproteins comprises the immunoglobulin superfamily (PVR/CD155, Nectin-1/CD111, Nectin-2/CD112, Nectin-3, and Nectin-4) called the Nectin family [2- 7]. Nectins have the properties of both homophilic and heterophilic cell adhesion [5- 8]. PVR engages with Tactile/CD96, Nectin-1 interfaces with Nectin-3 and Nectin-4, and Nectin-2 and PVR connect with Nectin-3 and DNAM-1/CD226. Nectin-4, whose expression is exclusive to the embryo, is the only nectin not extensively expressed in adult tissues [5], [10], [11]. Nectin-4 is considered as embryonic carcino-antigens, is reexpressed in breast cancer. It is a novel, highly useful biomarker for breast cancer. Additionally, Nectin-4 overexpression is a distinct indicator for prognosis in many cancer types. Numerous studies have shown that increased Nectin-4 activity is strongly connected to tumour incidence and progression in a variety of cancer types, although it is still unclear how Nectin-4 protein affects the initiation and progression of these cancers [12]. The mechanisms underlying, biological activities, and recent developments pertaining to Nectin-4 protein expression pattern in diverse cancer types are all outlined in the this review article. Nectin-4 overexpression is linked to a number of tumour development factors, including proliferation, vasculogenesis, dissemination, epithelial to mesenchymal transformation, DNA repair, tumour recurrence, and a worse prognosis in a number of cancer types. The significance of Nectin-4 in every conceivable facet of tumor development are comprehensively highlighted in this paper, as is the underlying mechanisms behind Nectin-4-regulated cancer growth [Table 1]. We have also highlighted the possible treatment approaches that aim Nectin-4 particularly [13].

2. Role of nectin-4 in carcinogenesis

2.1 WNT/β-Catenin signaling

According to experimental data, Nectin-4 uses the Pi3k/Akt axis of WNT/-Catenin signalling to promote breast cancer stem cell (BCSC) self-renewal. Data from in vitro, in vivo, ex vivo, and clinical pathology studies demonstrated that WNT/-Catenin signalling and breast cancer spread were associated with increased Nectin-4. In contrast, Nectin-4 amplification in null cells accelerated EMT and metastasis and also promoted WNT/-Catenin signalling via Pi3k/Akt axis, which in turn regulates cancer stem cell production. Nectin-4 deletion decreased EMT, invasion, metastasis, and the WNT/-Catenin pathway. Breast tumour metastatic lesions in the axillary lymph nodes and primary tumour tissues both showed increased Nectin-4, suggesting that this protein serves as both a BCSC marker and a breast cancer metastasis marker. The current research clearly shows that Nectin-4 is a BCSC marker and that it causes breast cancer [14], [15].

2.2 Angiogenesis

Nectin-4's involvement in carcinogenesis is supported by research, however its participation in tumour angiogenesis has not been thoroughly elucidated. Human umbilical vein endothelial cells (HUVECs) and highly metastatic carcinoma cells cells were used by some researchers to create a good angiogenesis model, and the role of nectin-4 in angiogenesis was comprehensively investigated. Additionally, they offered comprehensive in ovo, in vivo, and in vivo proof that nectin-4 promotes angiogenesis. Upon hypoxia, the ecto-domain of nectin-4, which particularly interacts with integrin-4 on endothelial cells, is shed into the surrounding stroma as a result of ADAM-17 expression mediated by metastatic breast cancer stem cells (mBCSCs). Instead of Phospho-Erk or NF- pathways, this association stimulates vasculature through the Src, PI3K, AKT, and iNOS pathway. Its function in angiogenesis has been confirmed by the activation and abrogation of a vasculogenesis cascade in vitro, in ovo, and in vivo when the nectin-4 ecto-domain is present and absent, respectively. Nectin-4 ecto-domain and integrin-4 connection disruption may therefore be a strategy for inhibiting mBCSC-induced vasculogenesis [16], [17].

2.3 Interaction with ErbB2 tyrosine kinase receptor

Several malignancies, including breast cancer, have known to express increased levels of the Nectin-4 cell adhesion protein and the ErbB2 tyrosine kinase receptor, which encourage the growth and spread of cancer cells. Researchers have demonstrated that nectin-4 cis-interacted with ErB2 and augmented its dimerization and activation, followed by the activation of the phosphoinositide 3-kinase-AKT signalling pathway for DNA synthesis, using the malignant human breast cell lines T47D and SUM190-PT, in which both nectin-4 and ErbB2 were positive. Nectin-4's third immunoglobulin-like domain connected with domain IV of ErbB2 in cis. This area is distinct from the trastuzumab-interacting region. However, it is present in the trastuzumab-resistant splice variants of ErbB2, p95-ErbB2, and ErbB2Ex16. These trastuzumab-resistant splice variants were additionally cis-interacted with by nectin-4, which increased the activation of the phosphoinositide 3-kinase-AKT signalling pathway for DNA synthesis [18], [19].

2.4 Hippo signaling-dependent SOX2 gene expression

Nectin-4, which is increased in a variety of cancer cells, interacts with ErbB2 and its trastuzumab-resistant splice variants, p95-ErbB2 and ErbB2Ex16, boosting DNA synthesis by PI3K-AKT signalling in cohesive cultures of malignant human breast T47D cells. Some researchers discovered that, in a suspension culture, nectin-4 and p95-ErbB2, but not nectin-4 and either ErbB2 or ErbB2Ex16, synergistically increased SOX2 gene transcription and tumor growth. Nectin-4 and p95-ErbB2 increased T47D cell division in a suspension culture, however this effect was reliant on SOX2 gene expression. Nectin-4 and either one of p95-ErbB2, ErbB2, or ErbB2Ex16 worked together to similarly increase the PI3K-AKT signalling in T47D cells, which is known to promote SOX2 gene expression. However, only nectin-4 and p95-ErbB2, not nectin-4 and either ErbB2 or ErbB2Ex16, together cooperatively improved the expression of the SOX2 gene. Only

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nectin-4 and p95-ErbB2 worked together to stimulate the Hippo signalling, according to thorough research. In this cell line, YAP suppressed the SOX2 gene expression; hence, the SOX2 gene expression was enhanced by the MST1/2-LATS1/2 signaling-mediated inactivation of YAP. These findings suggest that Hippo signaling-dependent SOX2 gene expression is cooperatively regulated by just nectin-4 and p95-ErbB2, boosting anchorage-independent T47D cell proliferation, and not by nectin-4 alone and either ErbB2 or ErbB2Ex16 alone [20], [21].

2.5 Cell adhesion

Cancer cells are exposed to unfavourable extracellular matrix surroundings at all phases of tumour development, and they must adapt in order to get around the growth restrictions brought on by the degradation of matrix anchoring. The cell adhesion molecule PVRL4 (poliovirus-receptor-like 4), also known as Nectin-4, was discovered initially during search for genes that allow cell division independent of matrix anchoring. By encouraging cell-to-cell adhesion and matrix-independent integrin 4/SHP-2/c-Src stimulation, PVRL4 encourages attachment independence. The PVRL4 locus commonly exhibits copy number increases in solid tumours, and some of these tumours exhibit localised amplifications. Researchers showed that PVRL4 is necessary for malignant breast cells to develop. Additionally, by preventing PVRL4-driven cell-to-cell adhesion using monoclonal antibodies, it is shown that this unique approach of targeted treatment can slow the growth of orthotopically implanted tumours in living organisms [22- 24].

2.6 Receptor for Recombinant Measles Virus (rMV-SLAMblind)

Triple negative (TN) breast cancer, whose cells are refractory to both hormone and Herceptin therapies and frequently cause relapse and dissemination, is one of the most resistant variant of breast cancer. To treat TN breast cancer, novel efficient therapies are required. Researchers have previously shown that the recombinant measles virus rMV-SLAMblind exhibits anti-tumor action against malignant breast cells. Furthur investigations on the efficacy of rMV-SLAMblind in the management of TN breast cancer showed positive results. Seventy-five percent of the TN breast cancer cell lines under study expressed nectin-4 on their surface, which is a receptor for rMV-SLAMblind. Nectin-4-expressing TN breast cancer cell lines were infected by rMV-SLAMblind, which drastically reduced the viability of half of the tested cell lines in vitro. Furthermore, intratumoral injection of rMV-SLAMblind inhibited the development of tumours in MDA-MB-468 and HCC70 xenografts. Researchers administered the luciferase-expressing-rMV-SLAMblind to MDA xenografted mice intravenously in order to evaluate the effectiveness of target therapy for metastatic breast cancer. The virus multiplied inside the tumour, which significantly slowed its development. The virus's safety was examined by injecting it intravenously into normal cynomolgus monkeys; this did not result in any signs like those of the measles. These findings imply that rMV-SLAMblind is a viable therapeutic option for the treatment of advanced and/or TN breast cancers [25- 28].

Table 1: Role of nectin-4 in carcinogenesis and the underlying mechanisms/pathways involved

S. No	Role of Nectin-4 in carcinogenesis	Underlying mechanisms/pathways
1	Stem cell self-renewal	Pi3k/Akt axis of WNT/-Catenin signalling
2	Angiogenesis	Integrin-4 disruption
3	Cell proliferation	Interaction with ErbB2 tyrosine kinase receptor
4	Cell proliferation	*Hippo signaling-dependent SOX2 gene expression*

5	Cell adhesion	matrix-independent integrin 4/SHP-2/c-Src stimulation
6	Tumour growth	*Receptor for rMV-SLAMblind*

3. Clinical significance

The TN and basal breast cancers are examples of tumour types that are linked to worse prognosis and strong nectin-4/PVRL4 mRNA expression. In multivariate analysis of TNBCs, high PVRL4 mRNA expression demonstrated independent adverse prognostic significance for metastasis free survival (MFS). Normal adult cells, including breast cells, did not display the nectin-4 protein expression. In 62 percent of TNBCs, membranous nectin-4 expression was noted and mRNA expression was strongly correlated with this. Researchers created an ADC (Antibody drug conjugate), called N41mab-vcMMAE, that has a monoclonal antibody against human nectin-4 coupled to monomethyl auristatin-E (MMAE). This ADC internalised nectin-4 and caused dose-dependent toxicity on breast cancer cell lines that expressed nectin-4 in vitro. It also bound to nectin-4 with high affinity and specificity. The effectiveness of this ADC in inducing quick, thorough, and long-lasting effects on nectin-4- expressing xenograft TNBC tissues, including primary lesions, metastatic tumors, and local recurrences, was seen to be dependent on dose- and nectin-4 protein expression levels by tumour cells. Nectin-4 is a novel, potential prognostic indicator as well as a highly specific and effective therapeutic target for ADC against TNBC [29- 32].

Some researchers used FACS (Fluorescence activated cell sorting) analysis and immunohistochemistry (IHC), in 78 primary cells and cell lines from various sources and 57 breast cancers respectively and investigated the expression of the Nectin-4 protein. Quantitative PCR was also used to assess mRNA expression of Nectin-4 protein. On panels of 45 sera from normal individuals, 53 sera from patients with non-metastatic breast cancer at diagnosis, and 182 samples from patients with metastatic breast cancer, serum nectin-4 was identified by ELISA and compared with CEA and CA15.3 markers. The conventional Chi-2 test was used to compare the distribution of serological and histological molecular markers and clinical characteristics. There was no evidence of nectin-4 in healthy breast epithelium. Nectin-4, on the other hand, was expressed in 61 percent of ductal breast cancer and in just 6 percent of lobular cancer. Nectin-4 expression was inversely related with the luminal-like markers ER, PR, and GATA3 and highly correlated with the basal-like markers EGFR, P53, and P-cadherin. Nectin-4 was seen to be expressed in most ER/PR negative tumours. Nectin-4 serum detection enhances the monitoring of metastatic breast cancer patients: 74 percent of these patients showed association with CEA/CA15.3/Nectin-4 compared to 67 percent with the association CEA/CA15.3. Levels of serum Nectin-4, a tumor growth indicator, correlated with the frequency of tumour dissemination. Additionally as measure of effectiveness of treatment, serum nectin-4 corresponds 90% of the time with improvements in clinical course [33], [34].

One study examined the relationship between Nectin-4 expression and patients' clinical outcomes for lymphnode negative, T1/T2 breast cancer. 197 individuals with primary unilateral breast cancer (T1/T2) who did not exhibit lymphnode involvement or distant spread constituted the research group. Using immunohistochemical tissue microarray analysis to measure the expression of the nectin-4 protein, the results were compared to clinical data using Kaplan-Meier curves and multivariate Cox regression analysis. 34 out of 197 cancers (17.3%) demonstrated Nectin-4 expression on cell membranes (m-Nectin-4), while 122 out of 163 tumours (74.8%) showed significant cytoplasmic expression of Nectin-4 (c-Nectin-4High). In Kaplan-Meier analysis, patients with a shorter disease-free survival (DFS) and distant relapse-free

survival (DRFS) rate had m-Nectin-4 positive. According to this multivariate study, m-Nectin-4 positivity in luminal-A cancers is a standalone predictive factor for DFS and DRFS but not for local relapse-free survival (LRFS). On the other hand, in patients with luminal-A tumours, c-Nectin-4 expression was substantially related with increased rates of DFS and LRFS but not DRFS. The m ultivariate study also revealed that the predictive significance of c-Nectin-4 low or nill expression in patients with luminal-A cancers is only restricted to DFS and LRFS. Hence it was proposed that in luminal-A type early stage breast cancer, nectin-4 serves as both a prognostic indicator and a target for molecular therapy [35], [36].

Another study investigated Nectin-4 expression in luminal B HER2 negative subtype breast cancer. 147 individuals with primary unilateral breast cancer and no signs of metastatic disease constituted the study group. Immunohistochemistry was used to measure the expression of the nectin-4 protein, and the results were compared to the clinical data by using Kaplan-Meier curves, multivariate and univariate stepwise proportional hazard assessment. Nectin-4 overexpression was inversely associated with overall survival, disease-free survival, and distant relapse-free survival with the same significance as it did with the size of the tumour. Nectin-4 and age, ER, PR, lymph node metastasis, tumour differentiation, histological subtype, and Ki-67 proliferation index did not show a statistically significant association [37].

Some researchers have shown how Nectin-4 is involved in lymphangiogenesis and controls it in invasive ductal carcinoma (IDC). Alcohol, smoking, unhealthy lifestyle choices, and other risk factors for breast cancer, as well as the expression of CXCR4 and the density of lymphatic vessels (LVD), were all positively connected with nectin-4 expression. In comparison to the initial tumour, LVD was considerably greater in metastatic lesions of the axillary lymph node. Nectin-4, VEGF-C, or both were depleted, which inhibited the expression of the crucial lymphangiogenic marker LYVE-1, tube formation, and migration of ALN derived primary cells. Under hypoxic circumstances, nectin-4 induced the expression of CXCR4 and CXCL12 in axillary lymphnode derived primary cells. Nectin-4 also increased the expression of matrix metalloproteases and indicators for lymphaticand metastatic disease, such as eNOS, TGF-, and CD-105. In lymph and blood circulating tumour cells of local and distant metastatic samples, Nectin-4 was shown to be induced together with other typical metastatic markers. Thus, via modifying the CXCR4/CXCL12-LYVE-1- axis, nectin-4 demonstrated a prominent role in driving tumor-induced lymphangiogenesis and lymphatic metastasis [17], [38].

4. Techniques for analysis of Nectin-4 expression

4.1 Immunohistochemistry (IHC)

Commercially available monoclonal anti-body, mAb (M22-244b3), against the ECD of E. coli–derived recombinant human nectin-4 is freely available. The antibody is generally validated for nectin-4 specificity by comparing staining patterns on processed tissue sections of various cell lines, xenograft tissues, and patient samples with available RNA data. The specificity of the antibody is also confirmed by Western blot analysis. IHC can be performed on frozen sections, tissue micro-arrays, and formalin-fixed, paraffin-embedded tissue sections. After deparaffinization and rehydration, tissue sections were treated for antigen retrieval with EDTA for 30 minutes and incubated with M22-244b3 the primary antibody or IgG1k isotype control antibody mouse for one hour. The colour on the immunostained slides is produced by tagging nectin-4 antibodies with either alkaline phosphatase (AP) or horseradish peroxidase (HRP). Nectin-4 displayed cytoplasmic and distinct membrane localisation in IHC. Given that Nectin-4 expression groups with basal markers, it is possible that a small subset of the progenitor cells from where these malignancies arise have Nectin-4 [39- 41].

4.2 Immunofluorescence

Tissue slices, individual cells, and cultured cell lines can all be studied using immunofluorescence. Researchers can identify which intracellular regions are expressing the antigen using immunofluorescence. Nectin-4 was found to be expressed in the cytoplasm and to have a distinct location at intercellular connections between cancer cells in immunofluorescence experiments that supported the results of IHC. Double immunofluorescence can also be performed to investigate coexpression of Nectin-4 with other tumour markers such as Nectin-4-p63 and Nectin-4-Laminin study in studying molecular features of normal and malignant cells, the knowledge of which can provide novel insights into molecular target therapy [42].

4.3 ELISA

In patients' serum and ascites samples, soluble nectin 4 may be measured using a sandwich ELISA. You can utilise tissue culture plates with 96 wells coated with mouse antihuman nectin 4 monoclonal antibody at a concentration of 10 µg/mL. 100 µL of serum or ascites can then be added to the wells after they have been blocked with PBS containing one percent bovine serum albumin, washed, and incubated for 12 hours at 4°C. Finally, 0.5 µg/mL of biotinylated goat antihuman nectin 4 antibody can be added. Bovine serum albumin and streptavidin-peroxidase should be incubated for an hour at 37°C. Following the addition of 100 µL of peroxidase substrate, the optical density will be measured at 405 nm. this should be followed by three to five rounds of washing in between PBS incubations containing 0.5 percent polysorbate 20. The nectin 4 concentration was calculated using serial dilution of recombinant human nectin 4-Fc protein [43], [44].

4.4 Quantitative PCR

Total RNA can be extracted from patient tissue samples using commercially available extraction kits. The 189-base-pair sequence corresponding to nectin 4 can be amplified with the corresponding forward and reverse primers. Initially, one-step RT-PCR was performed with the RT-PCR Access kit with temperature conditions as recommended by the kit manufacturer until final extension should be performed. Expression of nectin 4 in MCF7 breast cancer cell lines that has been reported previously can be used as a positive control. Real-time quantification of nectin 4 can also be performed using commercially available assay kits and the iQ5 Real-Time PCR thermocycler. Complementary DNA can be amplified by using nectin 4 forward and reverse primers. Following an initial denaturation step, several cycles of PCR can be performed under prescribed conditions recommended for denaturation and annealing/extension. Agarose gel electrophoresis can be used to determine the product size. The software used for iQ5 real-time detection allows for the calculation of threshold cycle values. By charting thresold cycle values against the log of the original beginning quantity of RNA in nanograms, standard curves for nectin 4 may be created [44], [45].

4.5 Flow cytometry

Cells can be incubated with mouse anti–nectin 4 at prescribed temperature conditions, which are then to be washed and, then incubated with biotin-conjugated goat antimouse IgG F(ab')2 fragment. After washing, cells are then incubated with allophycocyanin-conjugated streptavidin. After washing, cells are then resuspended and run on a flowcytometer cell analyser and analyzed using the manufacturer software, gating on live cells by forward and side scatter. Researchers who investigated the expression of nectin 4 in cancer cells have reported overexpression of the protein [44].

4.6 Serum Marker

Nectin-4 is a sensitive, credible, and complementing blood marker for the follow-up of patients with metastatic breast cancer, as it is shed from the tumour cell surface. Tumor necrosis factor-alpha-converting enzyme (TACE) breaks down nectin-4, and soluble nectin-4 is then released into the serum. Nectin-4 cleavage is significantly impacted by RNA interference, synthetic (TAPI-1) and natural (TIMP-3) TACE

inhibitors, and both. When a patient has metastatic breast cancer, the levels of serum nectin-4 frequently correlate with and fluctuate along with those of CEA and CA15.3. Serum Nectin-4 is frequently detected concurrently with CEA and/or CA15.3, which may indicate that the source of circulating Nectin-4 is not an indirect process like an immune response but rather comes directly from tumour cells. Using mouse anti-human Nectin-4 antibodies, sandwich-type ELISA can be used to determine the levels of nectin-4 in serum [46].

4.7 RNA interference assay

Small interfering RNA (siRNA) duplexes against the target genes to assess the biological roles of Nectin-4 in lung cancer cells can be used as described by [47]. The sequences that are a target for the synthetic oligonucleotides for RNA interference were as follows: control 1 (a nonspecific control oligo); control 2 (Luciferase: Photinus pyralis luciferase gene), 5′-CGUACGCGGAAUACUUCGA-3′; siRNA-Nectin-4-#1, 5′-ACAGUUACCACGUCUGAGGUU-3′, siRNA-Nectin-4-#2, 5′-AAUGGUUCAUGGCCUGUUUUU-3′.

4.8 Western Immunoblotting

Total protein must be extracted first by using commercially available cell lysate solutions customised for the purpose of western blot test and then stored at –80°C. About 50 micrograms of total cell lysate are separated on a 10% SDS Tris-hydrochloride polyacrylamide gel and then blotted onto a polyvinylidene difluoride membrane. Membranes can then be blocked with five percent powdered milk phosphate buffered saline, and then incubated in one µg/mL mouse antihuman nectin-4 overnight, followed by a two hour incubation in horseradish peroxidase–conjugated rabbit antimouse antibody diluted 1/5000. Protein is visualized using the commercially available Super Signal West Femto kit that enables detection of low femtogram amounts of protein deposited on nitrocellulose or membrane and probed with appropriate primary and secondary antibodies. Membranes are then exposed to autoradiography film and developed [44], [48], [49].

4.9 Northern blotting

Northern blotting is a method for determining the size and steady-state level of a specified RNA in a clinical specimen. The RNA is size-fractionated by gel electrophoresis and blotted onto a membrane to which it is covalently attached. The membrane is then hybridised to one or more particular probes that have been labelled for further detection. In the detection of nectin-4, the northern blot membranes have been hybridized with cDNA human probes and murine probes. Expression of nectin-4 can be analysed in various tissues using such northern blot membranes. Based on such studies it was found that nectin-4 is not expressed in normal breast tissue and found in embryological tissues. Any type of biological sample can be used for this technique but a disadvantage being unable to localize the specific site within the tissue [50], [51].

5. Scoring methods available for quantification of Nectin-4

5.1 IHC scoring

IHC assays are crucial for understanding tumours and evaluating biomarkers. IHC images are typically qualitatively assessed by a qualified professional, such as a pathologist, and occasionally this is supplemented by a semi-quantitative score. Nectin-4 exhibits a membranous expression in some TNBC tumors but is undetectable in normal adult tissue. Depending on the molecular type, nectin-4 positive expression might range from strong to weak. It is possible to utilise a digital image-based pixel H scoring system or the pathologist's ocular, manual quantitative scoring method to grade the expression of immuohistochemical marker [52].

5.2 Quick score

Many laboratories employ the Quick score, which takes into account both the percentage of cells and the degree of staining [Table 2]. The Quick Score results show good agreement with the biochemical tests and offer useful prognostic and predictive data. Therefore, the oncologist may more accurately anticipate the likelihood that a patient will respond to treatment by using a score system that semi-quantifies the expression of hormone receptors [53].

Table 2: Quick scoring of Nectin-4 protein expression by accounting both the percentage of cells and the degree of staining

Score	Intensity	Proportion of cells
2	2+ -strong positive	>50% of cell
1	1+ - weak positive	<50% of tumour cells
0	0 – no staining	No staining
Adding the two gives a maximum Quick score of 6.		

5.3 H score

In this scoring system, each intensity grade of the immunostaining is given an ordinal score, which is then multiplied by an estimated percentage of the tissue that is immunostained. The H-Score is computed as the sum of these numbers, and it ranges from 0 to 300 [54- 56]. Sample computation is shown in table 3.

Table 3 showing sample computation of Nectin-4 expression levels to produce H-score

Intensity score	0 -no staining	1 -weak	2- moderate	3 - strong	
Distribution (% cells or tissue stained)	0	20	30	50	H score = 0+20+60+150= 230
Scores (multiply rows 2 and 3)	0	20	60	150	

5.4 Quantitative pixelwise H score

Some labs utilize the pixelwise H score (pix-h score), which is among the most complicated. Individual cells and their nucleus, cytoplasm, and cell membrane are analysed based on the pixels and cells are classified as positive or negative based on the relative expression of the biomarker in one or more sub-cellular compartments. Based on the biomarker signal intensity, positive cells are further categorised as high (3+), medium (2+), or low (1+). The pix h-score is calculated as the weighted sum of the number of positive cells divided by the total number of identified cells. The pix H-score, which ranges from 0 to 300, captures both the intensity and proportion of the biomarker of interest from the IHC image [57], [58].

6. Conclusion

Nectin-4 is a novel tumour biomarker that has recently gained attention of cancer researchers thorughout the globe. Recent studies have shown that increased Nectin-4 activity is strongly connected to tumour incidence and progression in a variety of cancer types and yet underlying mechanisms involved in the carcinogenesis of Nectin-4 is still largely unknown. Ongoing researches are trying to understand the role played by Nectin-4 in order to use it as a target for chemotherapy of various solid tumours especially in poor prognostic tumours like TNBCs. In this brief review paper, we have summarised all the mechanisms that have been identified as roles played by Nectin-4 in carcinogenesis by various researchers so far. We have also summarised various methods of detection and scoring used by various researchers.

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