CORRELATION IN THE EXPRESSION OF HER2 RECEPTOR AND Ki-67 ANTIGEN IN MAMMARY ADENOCARCINOMAS IN BITCHES

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Abstract

Present study aimed at demonstration HER2 and Ki-67 antigen expression using immunocytochemical techniques and evaluation of the extent to which expression of the two markers correlates in adenocarcinomas of the mammary gland in bitches. In the course of surgery of mammary tumours, the material was sampled from 40 bitches of various breeds, aging 7 to 16 years. The tumours were verified histopathologically and immunohistochemical reactions were performed in order to detect HER2 and Ki-67. Microphotographs of the obtained preparations were subjected to computer-assisted analysis using MultiScanBase V 8.08 software in the Windows environment. The study of mammary adenocarcinoma samples demonstrated the presence of both HER2 receptors (72% cases) and Ki-67 antigen (62% cases). Comparison of the tumour samples demonstrated a strong positive correlation between expression intensity of the two neoplastic markers (correlation index r = 0.699).

Key words: bitches, HER2 receptor, Ki-67 protein, epithelial tumours, mammary gland.

Tumours belong to most dangerous diseases both in humans and in animals. In Poland similarly to the majority of developed countries of the world, mammary carcinoma represents the most frequently occurring female malignant tumours. Similarly in bitches, mammary gland tumours represent, together with dermal tumours, the most frequently manifested oncological lesions in the species (8).

The considerable progress of science in the branch of molecular biology, observed in recent years, allowed understanding several of the processes linked to tumour progression in the mammary gland. In the oncogenetic process significant roles are played by, for example, steroid-independent activation of oestrogen receptor (ER), its beta subtype, cyclooxygenase 2 (COX2) and receptors for epidermal growth factors (HER) (6, 14, 15). The latter ones, responsible for tyrosine kinase activity, are of particular interest. Their augmented expression was demonstrated in several tumours of both epithelial (3, 24) and mesenchymal origin (13). The receptors share common structure, consisting of 3 elements: the extracellular ligand binding domain, trans-membranous part and cytoplasmatic domain, manifesting activity of tyrosine kinase. The HER (human epidermal growth factor receptor) family includes 4 receptors: HER1, called also EGFR (receptor for epidermal growth factor – EGF), HER2 (protein p185), HER3 and HER4 coded by 4 proto-oncogenes: erbB-1, erbB-2 erbB-3 and erbB-4, respectively (17, 27). Individual types of the receptors are activated by various ligands and their common property involves the fact that following binding of the ligand the monomers of cell surface start to bind to each other, forming homo- and heterodimers. This allows the transactivation of HER 2 receptor within the heterodimer (HER2/HER1 or HER2/HER3) through HER1- and HER3-specific ligands which do not bind directly to HER2 (9, 25). Thus, despite the absence of a specific ligand, which has not been discovered till now (17), HER2 participates in signal transfer to the cell interior. It should be added that the presence of HER2 in a heterodimer causes that the structure exhibits a higher signal-transfering potential, as compared to the other dimers (9, 7).

All till now recognized types of receptors in HER family manifest little expression also in normal cells of adult body. However, amplification and/or overexpression of respective genes disturbs normal control of cell growth and differentiation inducing neoplastic transformation of the cells (2, 27). Multiplication of normal gene copy numbers in a cell in the presence of protooncogenes such as HER genes leads to oncogenic activation of the cell (16). Increased number of identical genes manifesting elevated expression leads to overproduction of the coded by
them proteins. In cases of amplified HER2 genes high amounts of p185 protein appear on the cell membrane. In such a situation the receptor function becomes clearly disturbed (27). In physiological conditions HER receptors transmit mitogenic signals originating from extracellular growth factors. High density of HER receptors on the cell membrane leads to massive formation of dimmers which start to send mitogenic signals independently of the extracellular signals. The mitogenic signals, transferred by the ras-raf-MAPKs pathway to cell nucleus, potently initiate cell cycle and intensify proliferation (19, 27). Thus, amplification of HER family genes leads to overexpression of the receptor family which, in turn, promotes the development in the host cells of a clearly malignant phenotype (16).

Evaluation of cell proliferation intensity by detection of typical proteins which appear in the cell cycle, e.g. of Ki-67, represents one of the ways in which aggressiveness of the tumour can be appraised. Ki-67 belongs to non-histone proteins present in cell nucleus. Its presence can hardly be detected in G1 phase of the cell cycle, increases in S and G2 phases to reach peak in M phase, which is followed by its abrupt disappearance in G0 phase. Time course of the protein expression results that it can be detected only in dividing cells (5, 12). The ratio of cells manifesting Ki-67 expression to the number of cells showing no such expression is termed the proliferative index, representing mitotic activity of tumour cells (4).

Present study was aimed at immunocytochemical demonstration of HER2 and Ki-67 expression and determination of the extent of correlation between the markers of mammary adenocarcinomas in bitches.

Material and Methods

Material for the study was sampled during surgical procedure from 40 bitches of various breeds, aging 7 to 16 years and manifesting a mammary tumours. The type of tumour was histopathologically verified.

Tumour samples were fixed in 10% buffered formalin, dehydrated and embedded in paraffin blocks. For the determination of HER2 receptor and Ki-67 antigen expression in paraffin sections of the examined tumours we took advantage of mouse monoclonal antibodies (clone PN2A, supernatant diluted 1:50 and clone MIB-1, supernatant diluted 1:100, respectively). When both antibodies were used in parallel, the antigenic determinants were unblocked in a microwave oven using antigen retrieval solution. Colour reaction was developed using LSAB2 kit and diaminobenzidine (DAB). All the reactions were accompanied by respective negative controls using the primary negative control kit. All the reagents and antibodies originated from DakoCytomation, Poland.

The obtained preparations were photographed and the microphotograms were subjected to computer-assisted image analysis using Axiophot microscope (Carl Zeiss) coupled to a computer. The measurements took advantage of MultiScanBase V 8.08 software, working in the Windows environment.

HER2 expression was evaluated using the semi-quantitative scale which took into account percentage of positive cells, as follows: no reaction (-); 1-10% - weak reaction (+); 11-25% - moderate reaction (++); >26% of positive cells- intense reaction (+++). Expression of Ki-67 antigen was evaluated also semi-quantitatively proportion by calculating the positive cells as follows: 0-5% - no reaction (-), 6-25% weak reaction (+), 26-50%, moderate reaction (++), above 50% intense reaction (+++). The results were subjected to statistical analysis using the STATISTICA PL software (StatSoft, Poland), employing the Spearman’s correlation approach.

Results and Discussion

Mammary carcinoma represented one of the first tumours in cells of which the relationship could be demonstrated between augmented expression of HER2 and the extent of malignancy of the process (16, 26). Significance of the receptor in carcinogenesis was corroborated by numerous studies which indicated that 10-40 % of mammary cancers in women demonstrate overexpression of the protein (9, 18, 22). In a few similar studies performed on mammary tumours in dogs the range of obtained results on HER2 expression was relatively extensive. Ahern et al. (1) examined 23 malignant tumours of the mammary gland in bitches and noted expression of HER2 in 74% cases, Dutra et al. (11) in 35% cases but Rungisipit et al. (23) in only 21% of the 47 examined tumours. In our own studies, including 40 adenocarcinomas, expression of HER2 receptor has been demonstrated in 72% of mammary tumours in bitches (Figs 1-2).

The extensive scattering of HER2 expression results obtained in human and in animal tumours reflects most probably the use of unstandardized techniques for the detection and evaluation of the expression extent of the marker (11). In our own studies, 37.5% of tumours have demonstrated HER2 expression evaluated at +, 25% at ++, 10% at ++++, while in 27.5% of samples no expression of the receptor has been demonstrated (Fig. 3).

In studies performed on mammary carcinoma in women the relation was documented between the intensity of HER2 receptor expression and proliferative potential of tumour cells. Vera-Roman et al. (28) examined 50 malignant mammary tumours and despite the relatively low expression of the receptor, detected in only 12% of tumors, demonstrated high proliferative activity (Ki-67) in every HER-positive case. Reverse results were obtained by Yamashita et al. (30). The authors detected HER2 overexpression in over 20% cases, and Ki-67 in almost 54% of the examined tumours but no significant relationship between expressions of the two markers could be demonstrated.
Fig. 1. Low intensity of HER2 receptor expression in mammary gland adenocarcinoma in a bitch. 400x

Fig. 2. High intensity of HER2 receptor expression in mammary gland adenocarcinoma in a bitch. 400x

Fig. 3. Distribution of HER2 receptor expression intensity in mammary gland adenocarcinomas in bitches.
Fig. 4. Low intensity of Ki-67 protein expression in mammary gland adenocarcinoma in a bitch. 400x

Fig. 5. High intensity of Ki-67 expression in mammary gland adenocarcinoma in a bitch. 400x

Fig. 6. Distribution of Ki-67 antigen expression intensity in mammary gland adenocarcinomas in bitches.
Despite this lack of correlation between expressions of HER2 and Ki-67, the authors noted evident positive relationship between HER2 overexpression and cumulation of p53 protein, noted in over 50% of malignant human tumours and produced in excess in cells with damaged DNA. The authors documented also positive correlation between levels of the protein and Ki-67 expression. Similar results were obtained by Nicholson et al. (21) on 105 mammary carcinomas but they could not show correlations between HER2 expression and intensity of cell proliferation (Ki-67).

In our own studies on Ki-67 antigen in mammary tumor in bitches (Figs 4-5), expression of the marker at the level of + or higher has been shown in over 62% of adenocarcinomas.

Among the examined tumours, 37.5% cases manifested Ki-67 expression at the + level, 17.5% at the ++ level, and 7.5% at the +++ level. In 37.5% samples no expression of the antigen could have been detected (Fig. 6).

The usefulness of the marker in veterinary oncology has been confirmed by Giziński et al. (12), who examined 60 mammary gland tumours in bitches and demonstrated positive correlation between high values of Ki-67 indices and low extent of cell differentiation, presence of metastases, poor general condition and short post-operative survival time. In our own studies, comparison between bitch adenocarcinoma samples has documented a strong positive correlation (correlation coefficient r = 0.699) between intensities of HER-2 and Ki-67 expressions (Fig. 7).

In analogous studies on mammary gland cancers in women Lukashina et al. (20) similarly have shown that high expression of HER2 correlated with elevated expression of Ki-67 antigen. The authors have observed also that tumours with overexpression of HER2 and Ki-67 were larger and manifested a more aggressive behaviour. Similar results have been obtained by Dowsett et al. (10), who have detected a significantly higher level of Ki-67 in HER-positive as compared to HER-negative tumours. Wang-Rodriguez et al. (29) examined 65 cases of mammary cancers and noted that overexpression of HER2 correlated with shorter post-operative survival time of the patients and with presence of metastases in regional lymph nodes. However, no such a correlation could be disclosed when expression of Ki-67 was compared to survival time. It should be mentioned that in our studies most frequent tumours have demonstrated expression of both HER2 (37.5%) and Ki-67 (37.5%) at the + level. On the other hand, when no HER2 receptor expression has been noted in the tumors, expression of Ki-67 has also been negative in over 80% cases. Similarly, when no Ki-67 has been present only 15% of tumours has manifested HER2 expression and the expression intensity has been low, not exceeding +. In over 22% of tumours neither HER2 nor Ki-67 expression could have been demonstrated.

Summing up it should be noted that overexpression of either HER2 or Ki-67 in mammary gland tumours of humans and animals represent prognostically unfavourable indices, also in cases when the two markers fail to correlate with each other. The demonstration of their presence in tumour cells and determination of expression level and reciprocal correlation between the two markers allows for closer recognition of tumour cell biology. Moreover, such studies allow to categorize mammary gland tumours in to groups by demonstrating specific expression of certain markers, which should promote designing more effective targeted therapies, linked to lower amount of side effects.
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27. Szacikowska E., Kozłowski W.: Receptory HER/Erbb w prawidłowym nablonku i w kancerogenezie. Współcz Onkol 2000, 1, 7-12.

