The characterization of the exposure to immune mediated apoptosis and the regulation of immune cytotoxic activity in the environment of a neoplasm and in decidua

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Abstract

Acquiring the immune-mediated apoptosis and the ability to regulate the cytotoxic immune response are the main phenomena playing fundamental roles in such situations as neoplasm survival and creation of immune tolerance during pregnancy. The aim of this study was to investigate these phenomena through the evaluation of metallothionein and RCAS1 proteins in neoplasm and its healthy environment (clear surgical margin), physiological conditions in placenta and its environment (decidua) and the comparison to non-neoplasmatic lesions originating from the environment (nasal polyps, endometriosis). We have shown that the growth of RCAS1 expression was simultaneous to the infiltration of activated immunological cells of tumor environment as well as decidua. The activity of immunological cells was in our study selectively suppressed. Metallothionein expression growth was also observed in healthy tumors stroma and in decidua probably in response to the growing cytotoxic activity and tumor spread. Alterations in RCAS1 and Metallothionein expression seem to be associated with local immune dysfunction in nasal polyps and endometriosis. In conclusion, the ability to compensate the growing cytotoxic immune response is physiologically observed in decidua, the lost of this ability in tumor environment might participate in the development of tumor spread.

ORIGINAL ARTICLE

1. Introduction

Immune tolerance

Immune tolerance is a continuous process conditioned by various factors including interaction between neoplasmatic cells/trophoblast cells and immune cells. It has been described a participation of 64 antigens in the process of ovarian cancer cells escape from host immunological surveillance [1]. Despite of the investigation on so many factors participating in tumor escape from immune surveillance, this process is still not clearly understood. The understanding of this process has to be reinforced by the tumor environmental behavior [2–4]. The result of neoplasm development is not only determined by the uncontrolled proliferation but also the disintegration of the origin tissue takes place.

The regulation of the number of cells is determined by apoptosis. Physiologically tissue can be remodeled by apoptosis as a response to external factors. In 1972 JF. Kerr observed the possibility of recycling of apoptotic bodies by adjacent normal cells [5]. The genes amplification originating from apoptotic bodies was confirmed [6]. DNA and mRNA were described to be separately packed in various apoptotic bodies what indicates the existence of biological material recycling through apoptosis [7]. It was also confirmed that a metastasis may develop from one single cell or even from mutated DNA originating from apoptotic body [8]. The effect of immune cells activity is normally the elimination of impaired cells through apoptosis; the apoptosis of neoplasmatic cell results in the development of apoptotic body containing mutated DNA which may also be used by normal cell and this way the phenomenon of apoptosis may be a step for tumor spread instead of the proper immune cells activity target. What is the biological reason of this process? Immune tolerance during pregnancy is not only the process of inhibition of immune response by trophoblast cells but also by the presence of apoptotic bodies originating from fetal cells in maternal circulation [9]. The usage of fetal genetic material (amplification) by mother was also confirmed [10,11]. It was also shown that fragments of these cells are necessary not only for the selective inhibition of maternal immune response, because these fragments express Fas-ligand (Fas-L/CD95-ligand/CD178), induces apoptosis of Fas (CD95/Apo-1) expressing cells [12] but also are responsible for the generating an adequate maternal cytotoxic response necessary for proper pregnancy development. The phenomenon of maternal immune tolerance during pregnancy should be understood not as a general inhibition of maternal immune system but rather as the selective suppression of activated cytotoxic cells. Since apoptotic bodies seem to play such an important role in the process of immune tolerance the location of their source (maternal-fetal interface and the margin of neoplasmatic tissue as well as tissues potentially able to use this cellular material) has to be taken into consideration. Similarly immune tolerance develops for the growing neoplasm, which can avoid immune recognition and evade immune mediated apoptosis despite the infiltration of activated immune cells- TIL (tumor infiltrating lymphocytes). Therefore the mechanisms responsible for the host toleration for to the high number of immune cytotoxic cells in the vicinity of tumor seem to be interesting. The dissemination of tumor cells (tumor spread through the environment) may be determined by the lost of tissue ability to compensate the cytotoxic immune response. Similar physiological process is observed during pregnancy. Decidualization is a process where the accumulation of cytotoxic active cells is observed [13]. The coexistence of endometrium and immune cells (integrity of tissue) is determined by the ability of endometrial cells to regulate cytotoxic activity and the resistance to immune mediated apoptosis. The highest concentration of immune cells is concomitant with ovum implantation and is responsible for the restriction of trophoblast cells invasion within decidua [14]. The interaction between endometrial cells and the immune cells without ovum implantation results in menstruation bleeding. When damaged ovum implantation occurs this interaction results in abortion [15–16]. Many factors of immune-modulating activity are known present in endometrium (Interleukin-15, Interleukin-13, Tumor growth factor-TGF, TNF-alpha, Interleukin-11, Fas, Fas-L and others) [17–20]. These factors are strongly secreted beginning with the implantation window through the whole pregnancy duration. The participation of decidual cells in the process of immune tolerance during pregnancy has not been precisely explained yet. The proportion of decidual and placental inhibiting activities in the maternal immune tolerance during pregnancy still remains unclear.

The immune tolerance during pregnancy has been compared to the phenomenon of immune tolerance to neoplasm [21] therefore we have performed molecular analysis of proteins expression responsible for the regulation of immune cytotoxic activity and the resistance to immune mediated apoptosis in the environment of trophoblast and neoplasmatic cells: decidua and clear surgical margin of malignant neoplasm. The expression of these factors was additionally evaluated in non-neoplasmatic lesions based on immune dysfunction: nasal polyps and endometriosis.

2. Results

The alteration of RCAS1 and Metallothionein expression regarding immune cells infiltration

RCAS1, which was until now thought to be responsible for tumor escape from immune surveillance in various human cancer cells [22,23] seems to be an important factor for cytotoxic activity regulation in the endometrium [24]. It has been reported to be expressed with high frequency in breast, lung, larynx and pharynx, gallbladder, ovarian, uterine, bile duct cancer cells, in Reed Sternberg cells and erythroid progenitor cells [25–31]. The RCAS1 protein acts as a ligand for a putative receptor present in various human cells including normal peripheral lymphocytes such as T, B, and NK (natural killer) cells

[22]. RCAS1 inhibits growth of receptor expressing cells in vitro and in vivo and induces apoptotic cell death through activation of caspase 3 and collapse of mitochondrial transmembrane potential [31]. The RCAS1 high expression in gallbladder, cervical and lung cancers increased with tumor stage and was linked to the depth of tumor invasion, to lymph node metastases, and was an independent unfavorable prognostic factor [25,26,32]. RCAS1 is however not only the marker of tumor progression. The expression of RCAS1 recognized in the bone marrow, Waldeyer's ring, placenta, endometrium, autoimmune liver disease and the tubal mucosa indicates its role in immune cells regulation [33–37].

Metallothionein (MT) is a cysteine-rich, low molecular weight cytoplasmic protein with functional roles in cell growth and differentiation [38]. MT immunoreactivity was prominent in cancer tissue, presenting a nuclear pattern of staining being inversely correlated with the apoptotic index [39]. It is also known that perinuclear MT location is important for protective function of MT against DNA damage and apoptosis [40,41], through the regulation of zinc-dependent enzymes and proteins activity. MT expression has been linked to reduced apoptosis in carcinoma cells [42,43,44]. MT participates in the regulation of DNA-repair process by PAPR-1 (the polyADP-ribose-polymerase-1) [45]. MT was found to interact specifically with subunit of NF-κB (nuclear factor κB) in breast cancer cell line, and inhibit the binding of NF-κB to DNA following TNF (tumor necrosis factor) activation [46]. Its expression was observed in the endometrium, in endometrial cancer and ovarian endometriosis [24,47–49]. MT expression seems to protect endometrial cells against apoptosis enabling them to acquire resistance to immune-mediated apoptosis [24]. MT expression is recognized as a useful prognostic tool, especially in invasive ductal breast carcinoma [50]. In head and neck cancer high MT immunoreactivity correlated with increased local and regional recurrence, resulting in poor prognosis [51].

2.1 Endometrium according to menstrual cycle changes

An increase of MT and RCAS1 expression was identified during the decidualization. The peak of MT and RCAS1 expression was observed during mid-secretory cycle phase. The higher MT and RCAS1 expressions were related to the growth of number of CD56 positive cells (natural killer cells) and the increase of CD69 (early activation marker of lymphocytes) expression [24,52]. In contrary, the growth of RCAS1 expression during the secretory cycle phase was associated with a decrease of CD25 (receptor for interleukin-2) expression [53].

2.2 Fallopian tube mucosa during implantation of ectopic pregnancy

Significantly more abundant CD56 and CD69 positive cells were observed in tubal mucosa during the tubal rupture in comparison to unruptured tubal ectopic pregnancy. In contrast to spontaneous abortion the growth of immune cytotoxic activity was not related to an adequate increase of MT expression in tubal implantation site. MT expression during tubal rupture in tubal mucosa has been significantly lower than in decidua during spontaneous abortion. In both cases the level of CD 56 and CD69 expression was comparable [54].

2.3 The initiation of labor

The maternal-fetal interface during the labor is typified by high cytotoxic activity [55,56]. The results of our research suggest that this growth comes from the drop of suppressive placental function which is observed during spontaneous beginning of the labor. Significantly lower RCAS1 expression has been observed during spontaneous vaginal labor in comparison to induced vaginal labor [35,57,58]. The placental RCAS1 expression was decreasing with the uterine cervix ripening during cesarean sections. Significant drop of RCAS1 has been observed when the cervix was ripen over 2cm [59]. The level of immune tolerance during the initiation of labor was not related to the fetal maturity [60]. The growth of cytotoxic response during the labor was related to the growth of MT expression in decidua [manuscript in preparation].

2.4 The retained placental tissue

The retained placental tissue observed during the third stage of labor may indicate that the process of placental detachment which is based on immune cells activity was not finished when the cervix was totally ripen. This was confirmed by the evaluation of placental RCAS1 level. The RCAS1 placental level was significantly higher in cases of retained placental tissue in comparison to spontaneous labor [61].

2.5 Endometriosis

Ovarian ectopic endometrial cells in our studies were typified by a decreased MT and RCAS1 expression levels with higher number of CD56, CD69 and CD25 positive cells in comparison to secretory endometrial cells [24,62]. This observation seems to confirm NK dysfunction described in endometriosis [63] as well as indicates the possibility of endometriosis development as a phenomenon secondary to the endometrium ability to coexistence with active immune cells. This interpretation remains in compliance with the observation of increased I-human lymphocyte antigens (HLA-I) expression in endometrial cells during retrograde menstruation which blocks NK mediated lysis [64]. In scar endometriomas (after cesarean section) MT expression as well as CD25 antigens expressions differed from that observed in ovarian endometriosis, while RCAS1, CD56 and CD69 antigens expressions remained at comparable levels [62]. These differences led us to a conclusion that the development of scar endometriosis may be reinforced by immune tolerance during pregnancy because it occurs more frequently after elective cesarean section [65]. Additionally the presence of RCAS1 positive macrophages were identified only in ovarian endometriosis, they were not present in scar endometriosis or secretory endometrium.

Therefore the physiological process of immune tolerance started during decidualization might participate in persisting of scar endometriomas cells [62,65,66].

2.6 The development of local lymph nodes metastases

Higher MT expression was found in breast cancer and head and neck cancer stroma in cases with the presence of local lymph nodes metastases in comparison to cases without lymph nodes metastases [67]. RCAS1 expression was also observed to be higher in head and neck cancer stroma in patients with the presence of lymph nodes metastases in comparison to patients without metastases [32]. This relation was not observed in breast cancer. The predominant cells among TIL in breast cancer were CD3 positive cells while no CD56 positive cells were observed. The number of CD3 and CD25 positive cells was significantly lower in the group of patients with the presence of lymph node metastases in comparison to patients without metastases although no differences in CD69 antigen expression were observed between these two groups of patients [29,68].

2.7 Nasal polyps

Nasal polyps are a benign growth of nasal mucosa based on immune mediated dysfunction [69,70]. In histopathological examination nasal polyps were in our study divided regarding the predominant cells infiltration and three groups of polyps were indicated: eosinophilic nasal polyps, neutrophilic nasal polyps and lymphocytic nasal polyps. Within these groups of polyps different resistance to immune mediated apoptosis was observed and an ability to regulate the immune cytotoxic response assessed by the expression of RCAS1 protein was noticed in nasal polyps. The higher RCAS1 expression was observed in lymphocytic nasal polyps in comparison to eosinophilic and higher in eosinophilic than in neutrophilic nasal polyps. Moreover RCAS1- positive macrophages were shown in the nasal polyps stroma where increased immune infiltration was present in pseudocystic structures [71]. Alterations in the resistance to immune mediated apoptosis enabling the polypoid tissue growth as well as the ability to regulate the activity of infiltrating immune cells were observed with respect to the type of immune cells infiltration.

3. Comment

The regulation of TIL activity in tumor adjacent tissue is currently under consideration [72,73]. Chang has presented the results of analysis of killer inhibitory receptors (KIRs) expression on TIL in human endometrial cancer and indicated that tumor might itself provide the increase of expression of NKG2A/CD94 on TIL which results in the restriction of their activity [74]. Similarly Ponte has noticed NKG2a expression alterations in peripheral blood NK cells derived from pregnant women and indicated that these changes result from the activity of trophoblast cells [75]. The participation of KIRs especially through the interaction with HLA-G1 in immune tolerance during pregnancy is well known [76]. The observed decreased expression of the IL-2 receptor in TIL in tissue adjacent to the tumor in breast and cervical cancers has been interpreted by Sheu as a result of existence of TIL selective suppression phenomenon [77]. Our results remain in concordance with aforementioned and selective suppression in our studies seems to be higher in patients with the presence of lymph node metastases. We posit that RCAS1 might be a factor responsible or facilitating the selective suppression phenomenon in tumor environment. Similarly the phenomenon of this selective suppression is observed during decidualization and seems to be a part of immune tolerance during pregnancy. The growth of CD69 antigen expression in secretory endometrium co-existing with decrease of CD25 antigen expression was identified in our study and remains in compliance with Saito and Ho results [78,79]. Saito indicated that these lymphocytes during secretory cycle phase remain rather more activated than in a resting state although CD25 expression was found to be lower in this study [79]. This might easily be explained by selective suppression phenomenon using RCAS1 expression. In sum, the selective suppression phenomenon is necessary for the preservation of the activated lymphocytes in decidua necessary for the restriction of trophoblast invasion assuring the inhibition of immune response against decidual cells. This phenomenon is necessary also for the assuring of tissue integrity during growing cytotoxic response against tumor cells and the loss of this ability may lead to tumor spread.

The interaction between stroma/decidua and immune cells is also observed in immune mediated diseases. In both endometriosis and nasal polyps derived from the environment of these lesions the presence of RCAS1 positive macrophages was observed. RCAS1 positive macrophages have been only observed in bone marrow where they were proved to participate in the maturation of progenitor cells [31] or in Hodgkin disease [80]. Recently, RCAS1 positive macrophages were described in immune mediated liver diseases [81]. The possible participation of RCAS1-positive macrophages in the etiology of immune-mediated diseases may indicate the importance of selective cytotoxic suppression phenomenon.

The results presented above may confirm the similarity of cancer environment to decidua. The key way to differentiate between these two phenomena is their reversibility in pregnancy during the labor. Although the inhibition of maternal immune response by placenta disappears, an adequate immune cytotoxic activity has to be assured and is maintained by the suppressive decidual cells activity. The growth of the resistance to immune mediated apoptosis occurs and is necessary for the coexistence of immune cells with decidual cells. The increase of MT expression has been observed in our study within decidual cells during spontaneous abortion and preterm births however, the lack of MT expression increase concomitant with growing cytotoxic response within tubal mucosa has resulted in tubal rupture. The tumor environment is typified by the MT overexpression

which may be the demonstration of physiological local processes regulation destruction.

In conclusion, selective suppression of active immune cells and resistance to immune mediated apoptosis in decidua are a part of immune tolerance during pregnancy phenomenon and are also observed in tumor environment. The loss of the ability to compensate the growing cytotoxic immune response in the environment might participate in the development of tumor spread.

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