Histological and epigenetic analyses of placenta tissue from breast cancer patients and healthy participants

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P 4-21-06

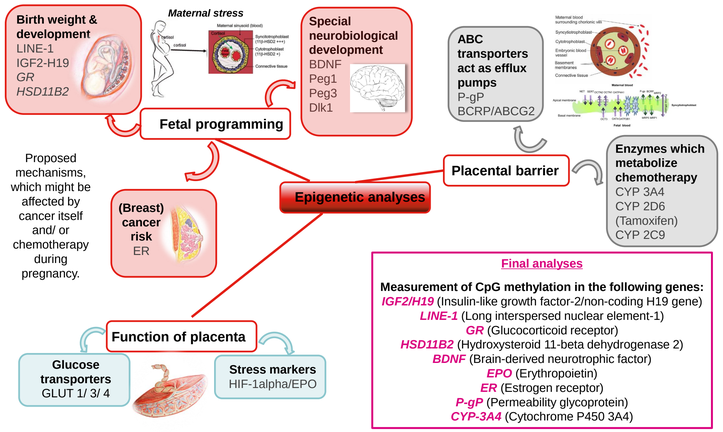
Background

Breast cancer is one of the most common malignancies during pregnancy.[1] Current guidelines demonstrated that breast cancer during pregnancy can be treated similarly to non-pregnancy-associated breast cancer, except for hormonal and anti-HER2 therapies.[2] Nonetheless, a decreased birth weight is often observed in newborns.[3] Therefore, this project aims to analyze the effects of chemotherapy and the impact of cancer progression on the placenta.

Patients and Methods

Placentas from breast cancer patients (n=66) and non-cancer participants (n=20) enrolled in Breast Cancer in Pregnancy (BCP) registry were collected after delivery and embedded in paraffin. Sections were stained with Hematoxylin-Eosin (HE) and IHC (Ki-67, cPARP, p27Kip1); trophoblast morphology was evaluated by an established scoring system including 5 criteria to reflect impairment of placentas. The distribution of immunohistochemical markers was semiquantitatively determined. All assessments were done by two independent investigators. Additionally, epigenetic analyses/cytosine methylation of genes shown in Fig. 1 were performed. These genes are involved in the following biological processes: Birth weight (*IGF2/H19*), global methylation (*LINE-1*), hypoxia (*EPO*), neurobiological development (*BDNF*), glucocorticoid binding (*GR*), inactivation of glucocorticoids (*HSD11B2*), breast cancer (*ER*), drug transport (*P-gP*) and drug metabolism (*CYP-3A4*).

Figure 1. Epigenetic analyses



Final analyses

Measurement of CpG methylation in the following genes:
IGF2/H19 (Insulin-like growth factor-2/non-coding H19 gene)
LINE-1 (Long interspersed nuclear element-1)
GR (Glucocorticoid receptor)
HSD11B2 (Hydroxysteroid 11-beta dehydrogenase 2)
BDNF (Brain-derived neurotrophic factor)
EPO (Erythropoietin)
ER (Estrogen receptor)
P-gP (Permeability glycoprotein)
CYP-3A4 (Cytochrome P450 3A4)

Results

Table 1. Demographic and delivery characteristics

Parameter	Category	BCP patients N=66	Non-cancer participants N=20	p-value
Age at delivery, years	Mean	33.9	31.9	0.061
	StD	4.1	4.5	
Week of delivery	Mean	37.1	39.1	<.001
	StD	2.1	1.1	

Table 2. Tumor characteristics of BCP patients

Parameter	Category	BCP patients N (%) N=66
Trimester of pregnancy at breast cancer diagnosis	First trimester	6 (9.1)
	Second trimester	31 (47.0)
	Third trimester	29 (43.9)
ER/PgR	Both ER, PgR negative	36 (54.5)
	ER and/or PgR positive	30 (45.5)
HER2 status	Positive	18 (28.1)
	Negative	46 (71.9)
Tumor grading	G1	1 (1.5)
	G2	22 (33.8)
	G3	42 (64.6)
Histological tumor type	Ductal invasive	56 (90.3)
	Lobular invasive	1 (1.6)
	Inflammatory	0 (0.0)
	Other	5 (8.1)

Table 3. Therapy during pregnancy and after delivery

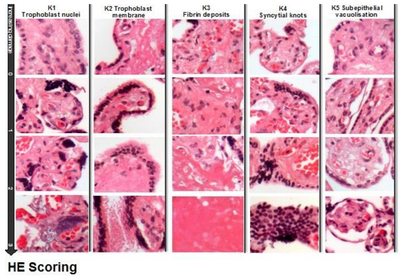
Regime	BCP patients N=66, N (%)
EC/AC	4 (6.6)
FEC/FAC	10 (16.4)
EC/AC followed by taxane	**32 (52.5)**
FEC/FAC followed by taxane	5 (8.2)
AC plus Docetaxel (combination)	5 (8.2)
Non-taxane plus taxane (combination)	1 (1.6)
Other chemotherapy	4 (6.6)

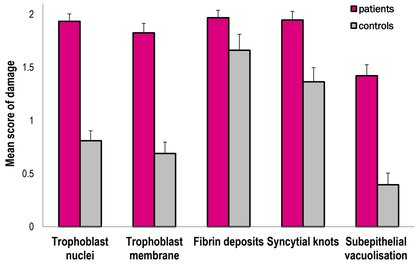
Table 4. Supportive therapy

Supportive therapy	Category	BCP patients N=45, N (%)
Dexamethasone	no	4 (9.3)
	yes	**39 (90.7)**
	missing	2
5-HT3 antagonist	no	7 (17.1)
	yes	**34 (82.9)**

Breast cancer patients were older and delivered earlier than non-cancer participants (Tab.1). Pregnant breast cancer patients received mostly epirubicin/ adriamycin in combination with cyclophosphamide followed by taxanes (Tab.3). Dexamethasone and 5-HT3 antagonists were often used as supportive therapy (Tab.4).

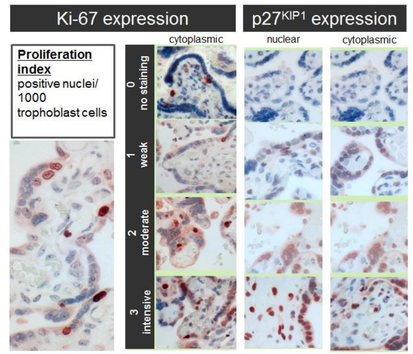
Figure 2. Histology HE Scoring

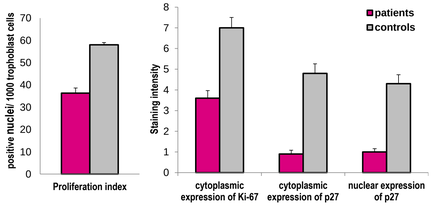




HE staining revealed significant damage of trophoblast nuclei and membranes in placentas from breast cancer patients compared to controls (Fig.2 , mean score of damage 1.9/1.8 vs 0.8/0.7, p<0.001).

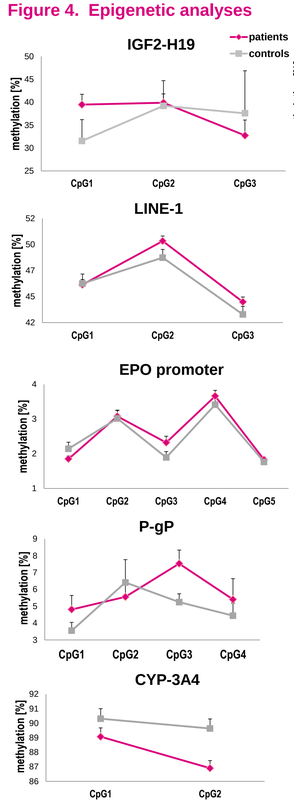
Figure 3. Immunohistochemistry





Mean proliferation index was reduced (Fig.3, 36.3 vs 58.0, p<0.001). Nuclear and cytoplasmic expression of the negative cell cycle regulator p27Kip1 was reduced (mean IRS score 1.0/0.9 vs 4.3/4.8, p<0.001). No evidence of enhanced apoptosis was found. Epigenetic analyses showed significant differences in mean cytosine methylation of *EPO* (68.4% vs 71.1%, p<0.05) and *CYP-3A4* (87.8% vs 90.0%, p<0.01) genes (Fig.4). Altered methylation of CpG positions of *LINE-1*, *IGF2/H19*, *HSD11B2*, *ER* and *P-gP* genes were found.

Figure 4. Epigenetic analyses



Conclusions

Placentas from breast cancer patients seem to be harmed in contrast to placentas from normal pregnancies, shown by morphologic abnormalities and a decreased proliferation index. Nevertheless, no increase of apoptotic cells could be demonstrated. Altered expression of efflux pumps or drug-metabolizing enzymes might be a reason for good fetal tolerability of chemotherapy during pregnancy as methylation patterns were changed in *P-gP* and *CYP-3A4* genes.

References

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Presented at: San Antonio Breast Cancer Symposium® -December 10-14, 2019

Karolin Fröhlich received a grant from the evangelic Scholarship Department Villigst.
The project was financially supported by the Wilhelm Sander Foundation.