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Fluctuation of intratumor biological variables as a function of menstrual timing of surgery for breast cancer in premenopausal patients

Despite the numerous studies performed, the best timing of surgery for premenopausal patients with breast cancer remains a debated issue due to its controversial association with clinical outcome. In particular, a renewed interest has grown in the clinical aspects of the hypothesis that the hormonal milieu at the time of surgery may influence patient prognosis [1], with the luteal phase probably associated with a decreased risk of relapse. Also, disease recurrence and survival might be affected by the menstrual timing of ablative/additive endocrine manipulations, with a favorable outcome of women undergoing ovarian ablation plus tamoxifen treatment during the luteal phase [2].

Based on previous findings that aggressiveness and invasion-related markers tend to be overexpressed in primary tumors removed during the follicular phase [3], and on the hypothesis that during specific times of the menstrual cycle up-regulation of hormonally controlled genes might influence tumor cell shedding and survival, we investigated the expression of biological variables involved in cell cycle and apoptosis regulation (p53, $p27^{kip1}$, Bcl-2, Bax) and angiogenesis [vascular endothelial growth factor (VEGF)] in breast cancers from 629 premenopausal women, as a function of the menstrual timing of surgery. All the women had a regular menstrual cycle not exceeding 36 days. The estrogen and progesterone receptor (ER, PgR) status of all tumors was biochemically determined. Expression of p53, $p27^{kip1}$, Bcl-2 and Bax was immunohistochemically evaluated and VEGF immunoenzymatically measured.

Regardless of ER status, PgR and VEGF concentrations fluctuated during the menstrual cycle (Figure 1 and Table 1), with the highest values around day 11. This was probably due to the stimulatory effect of increasing plasma levels of estradiol, which, after binding ER, induced the synthesis of these two estrogen-related proteins. In the subsequent luteal phase, PgR and VEGF concentrations dramatically decreased. VEGF expression paralleled estradiol levels and increased again during the late luteal phase concomitantly with progesterone withdrawal. In contrast, expression of $p27^{kip1}$, p53, Bcl-2 and Bax remained relatively stable, in agreement with previous results [4].

Among the biological explanations for the benefit of surgical and endocrine manipulations during the luteal phase of the menstrual cycle, one of the most accredited is the possibility that tumor eradication may minimize angiogenesis stimulation if scheduled when progesterone concentration is elevated and VEGF conversely decreased. However, there is no general consensus on VEGF concentrations during the menstrual cycle: published studies, always dealing with plasma or serum levels and mostly determined in healthy volunteers, have variably reported increased VEGF levels during the follicular or luteal phases, or no consistent change [5]. Our data on the fluctuation of VEGF expression in breast cancer confirmed the hypothesis of an inverse association with progester-

Table 1. Fluctuation of intratumor biological variables according to menstrual cycle phases

	Early follicular[a], days 1–7 (n = 100[b])	Late follicular, days 8–14 (n = 152)	Early luteal[a], days 15–21 (n = 159)	Middle luteal, days 22–28 (n = 133)	Late luteal, days 29–36 (n = 85)
PgR (fmol/mg protein)[c]	92 (1–1612)	125 (1–2510)	69 (1–1476)	73 (1–1305)	65 (1–1337)
VEGF (pg/mg protein)[c]	73 (6–218)	132 (9–246)	61 (9–175)	38 (13–247)	92 (10–294)
p53 (>5% positive cells)[d]	17	19	23	25	25
Bcl-2 (>30% positive cells)[d]	53	47	47	58	46
Bax (>10% positive cells)[d]	70	70	74	65	77
$p27^{kip1}$ (<10% positive cells)[d]	77	60	65	61	61

Progesterone receptors (PgR) and vascular endothelial growth factor (VEGF) were measured as for Figure 1. The expression of p53, Bcl-2, Bax and $p27^{kip1}$ was imunohistochemically determined.

[a]Menstrual cycles for each patients were divided into follicular and luteal phases on the basis of the date of the last menstrual period before surgery. Follicular phase patients were defined as those reporting a last menstrual period 1–14 days prior to surgery; luteal phase patients were defined as those reporting a last menstrual period 15–36 days before surgery.

[b]Information was not available on the full number of cases.

[c]Median values (range).

[d]Percentage of positive cases.

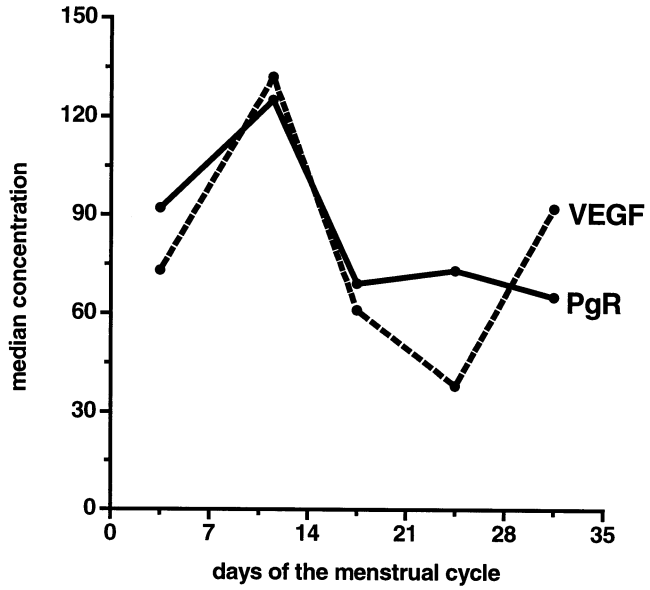

Figure 1. Fluctuation of intratumor progesterone receptors (PgR) and vascular endothelial growth factor (VEGF) according to menstrual cycle phases. PgR content was determined by a ligand binding assay and expressed as fmol/mg of cytosolic protein, and VEGF was measured by a quantitative enzyme immunoassay and expressed as pg/mg of cytosol protein.

one levels during the menstrual cycle, and demonstrated, for the first time, lowest intratumor VEGF values during the luteal phase, even regardless of ER status (data not shown). This finding might contribute to an explanation of the favorable outcome for women undergoing surgery, or whose hormonal treatment was initiated, during the second part of the menstrual cycle [2].

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