REVIEW ARTICLE

A SYSTEMS BIOLOGY APPROACH TOWARDS UNDERSTANDING THE PROCESS OF BLASTOCYST IMPLANTATION

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Abstract : Time-synchronous development of endometrium and embryo under adequate progesterone dominance is considered integral to the process of blastocyst implantation in the human. It now appears that hypothesis-driven and candidate-based deductive approach fails to explain this complex control process. We propose a systems biology approach to elucidate the control process underlying the physiological basis of successful interfacing between embryo and endometrium towards blastocyst implantation. Elucidation of the time course pattern of transcriptomics involved in the process of blastocyst implantation in mid-luteal phase endometrium with and without progesterone dominance, as well as, with and without viable embryo shall elaborate upon the polygenic and multifactorial nature of the process of blastocyst implantation. Accordingly, a large scale homeodynamic model of hierarchical arrangement of functional networks of regulatory genomic expressional elements at the level of endometrial receptivity shall emerge. It is anticipated that such a systems biology approach shall provide an integrated picture of the process and shall also open up novel areas of basic, strategic and translational research in the biology of blastocyst implantation.

Key words : endometrial receptivity   progesterone antagonism
       primates   transcriptomes   systems biology

INTRODUCTION

A linear application of simplistic, largely deterministic and logically reliable model of operations has, in past, held the notion that three primary modules are integral to the physiological process of blastocyst implantation in the human: endometrial competence, adequate progesterone priming, and viable endometrium – embryo dialogue (1, 2). However, blastocyst implantation is not a highly programmed process; it is rather a complex physiological process. Like any complex biological process, blastocyst implantation is dependent on non-linear interaction of multiple modules and motifs with contexts specific to time and space. These operational characteristics collectively

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attribute to the emerging property of a robust order in the system (3). Our knowledge about the process underlying the control system of blastocyst implantation is very thin (4). In the present essay, we propose that a large scale time course study of transcript profiles in adequately hormone primed competent endometrium in the presence of age- and stage-matched synchronous and viable embryo may divulge the nature of interaction networks of transcriptomes and their factorial regulation. We believe that deduction of such a large scale expressional networks dynamics shall provide a better picture of the process and possibly open up novel areas of basic, strategic and translational research in blastocyst implantation. In the present essay, we also provide the proof of principle by using deductively selected gene products based transcript profiles in receptive endometrium of the rhesus monkey.

Cartesian model of blastocyst implantation

According to the Cartesian paradigm, blastocyst implantation in the human is a process that is determined by linear and direct interaction of three major factors. They are endometrial competence, adequate progesterone priming, and endometrium – embryo dialogue. According to the notion, progesterone priming allows endometrium to acquire sufficient secretory maturation in a self-limiting manner. Thus, luteal phase progesterone plays the role of a prime mover (ie., primary locomotive module) for endometrial receptivity to blastocyst implantation. In case, time synchronous embryo-derived or analogous input-set is not available, primed endometrium breaks into menstrual loss (5). On the other

hand, progesterone dependent secretory maturation makes uterine luminal environment hospitable to support the growth and differentiation of preimplantation stage embryo. Time synchronous interaction between endometrium and embryo renders fine adjustments of endometrial receptivity allowing for blastocyst implantation (1, 2). A few paracrine factors have been shown to be involved in this scheme of action. The details of the schema have been given elsewhere (2).

Despite its simplistic and deterministic edges, the major problem of this model is that it fails to accommodate the following observations like :

(i) occurrence of successful implantation despite relative progesterone starvation (6),

(ii) occurrence of delayed implantation (7),

(iii) a subset cases of implantation failure with no apparent anomaly in endometrium and ovarian functions (8), and

(iv) generally low implantation rate in *in vitro* implantation and embryo transfer (IVF-ET) (7, 9).

We believe the above-mentioned phenomena may be addressed only when we start understanding the physiological complexity of the control process functional towards blastocyst implantation. It is becoming increasingly evident that a knowledge base of gene expressional networks in endometrium is a pre-requisite to elucidate the nature of the above-

mentioned control process of blastocyst implantation (10).

There are indeed a few human reports wherein transcripts profile of mid-luteal stage endometrium has been compared with that of proliferative stage or early luteal stage endometrium to delineate the role of progesterone in establishing endometrial receptivity for embryo implantation (11–17). Table 1 gives a summary of the reported studies. Albeit interesting data emerged from these studies, this model suffers from following critical limitations.

(1) These studies did not address the time course of endometrial trancriptomics during pre- to peri-implantation window.

(2) The potential impact of pre-implantation embryo derived signal on the transcriptomics of progesterone dominated endometrium has not been explored in these studies.

(3) These studies did not explore endometrial transcriptomics during window of implantation subjected to relative progesterone starvation by effective progesterone antagonism in pregnancy cycle. It has been earlier demonstrated that mifepristone administered at a relatively small dose (2 mg per kg body weight) in the early luteal stage rendered endometrium phenotypically non-receptive during the implantation window resulting in implantation failure without any discernible change in serum concentrations of estradiol and progesterone (18, 19).

Collectively, there has been no substratum for any serious attempt in the past to employ systems biology approach to elucidate endometrial receptivity in the human.

Multi-modular study design to delineate regulatory transcriptome networks

Therefore, we propose to perform experiments for a comparative analysis of transcript profiles in implantation stage endometrium and construct the time course dynamics of expressional networks operational in the process of endometrial receptivity. Since this study design cannot be applied in human subjects for ethical, practical and technical constraints, we employed rhesus monkeys as the primate model. In brief, expressional transcriptomics using cDNA based arrays for both ~400 and ~1200 separate gene products in endometrial samples collected from fecund cycles of rhesus monkeys on days 4 and 6 after ovulation with and without retrievable synchronous viable embryo (morula to blastocyst stages) and with or without mifepristone (2 mg per kg body weight) treatment on day 2 after ovulation was conducted (Table I). All the gene products selected were assumed to be involved in endometrial physiology and pathophysiology based on available reports. We have reported for the first time that an overt differential regulation of a set of genomic expression in implantation stage endometrium under adequate progesterone action and in the presence of stage- and age-synchronized embryo is an integral to the process of blastocyst implantation (20–22).

TABLE I : Previous array-based studies of endometrial receptivity in human and monkey.

Reference	Subject (No. of gene products)	Array experiment design (Sample size)
Kao et al. 2002 (11)	Normally cycling human (~12,000)	Between mid-proliferative (n= 4) and mid-secretory (n=7) phases
Carson et al. 2002 (12)	Normally cycling human (~12,000)	Between early (n=3) and late (n=3) secretory phases
Borthwick et al. 2003 (13)	Normally cycling human (~60,000)	Between mid-proliferative (n=5) and mid-secretory (n=5) phases
Catalano et al. 2003 (45)	Human endometrial explant culture (~1000)	Mid-secretory phase (n=5) endometrial explants treated with 10^{-9} M E_2 plus 10^{-7}M medroxyprogesterone acetate and 10^{-9} M E_2, 10^{-7} M medroxyprogesterone acetate plus 10^{-6} M RU486
Riesewijk et al. 2003 (14)	Normally cycling human (~12,000)	Between paired samples (n=5) collected during early and mid secretory phases
Ace and Okuliz 2004 (46)	Ovariectomized hormone simulated rhesus monkeys (~12,000)	Normal proliferative, day 13 (n=3) and mid secretory days 21 (n=3) and day 23 (n=3)
Ghosh and Sengupta 2005 (20)	Cycling rhesus monkeys (~400 custom made)	Fecund receptive versus fecund non-receptive (following RU486) on day 6 after ovulation (n=12)
Talbi et al. 2006 (17)	Normally cycling human (~55000)	Mid-late proliferative (n=5), early (n=3), mid (n=8) and late (n=6) secretory phases of cycle
Catalano et al. 2007 (47)	Normally cycling human (16000)	Mid-secretory endometrial samples with no treatment (control; n=15) and with mifepristone (200 mg) treatment (n=9)
Sherwin et al. 2007 (48)	Adult female baboons (8000)	Mid-secretory phase samples with either no treatment (control; n=2) or rhCG (1.25 IU/h) treatment (n=2) during days 5-10 after ovulation
Ghosh et al. 2009 (21)	Cycling rhesus monkeys (~400 custom made)	Fecund receptive versus fecund non-receptive (following RU486) on days 4 and 6 (n=28) after ovulation
Najwa 2009 (22)	Cycling rhesus monkeys (~1200 custom made)	Fecund receptive versus fecund non-receptive (following RU486) on days 2-8 (n=72) after ovulation

Transcriptomic pattern in the implantation stage receptive endometrium

Based on our cDNA based array results (Table I), we have constructed predictive pattern of transcriptomes using annotated structural characteristics of transcripts that displayed more than two fold changes in significant analysis of microarray (*http://www-stat.stanford.edu/~tibs/SAM*) in implantation stage endometrium relative to the one that has been subjected to progesterone starvation by low dose early luteal phase mifepristone rendering it inhospitable for embryo to implant, and to zero-embryo sojourn, respectively. The underlying assumption was that all the three conditions, namely progesterone adequacy plus zero-embryo (group 1), progesterone adequacy plus synchronous embryo (group

2), and progesterone starvation plus synchronous embryo (group 3) yield different process products (23) at the endometrial transcript networks level, and that the transcript networks connectivity and assortments follow explicit principle of mathematical engineering (24–26). It is also assumed that any one of the processes can be targeted to analyze (predict) using other two processes as probes (predictons) under the above-mentioned format (27, 28). It was also assumed that the predictive analysis follow the basics of Shannon informatics including the modules like signal, noise and hidden layer, as well as, the principle of entropization of the underlying coding process (29). It means that the coding process in the given issue of the control process of blastocyst implantation relates to the form and size of endometrial transcriptomes quantitatively, and is not necessarily related to corresponding and proportionate proteome products.

We report here that endometrial transcriptomes show reduction of gain in expressional variability in the presence of stage synchronous, viable embryo as evidenced by marked loss of differentially displayed gene expression between group 2 and group 1 endometrium on day 6 after ovulation [15] as compared to that on day 4 after ovulation [640] in ~1200 gene product arrays (Fig. 1). It is generally believed that lowering of gain is a way by which a complex coding process enhances its determinism, albeit at the cost of inherent vulnerability. As shown in Fig. 3A, networks analysis of 1200 gene products data also revealed a small-world type pattern of transcript connectivity in the presence of adequate progesterone plus viable embryo
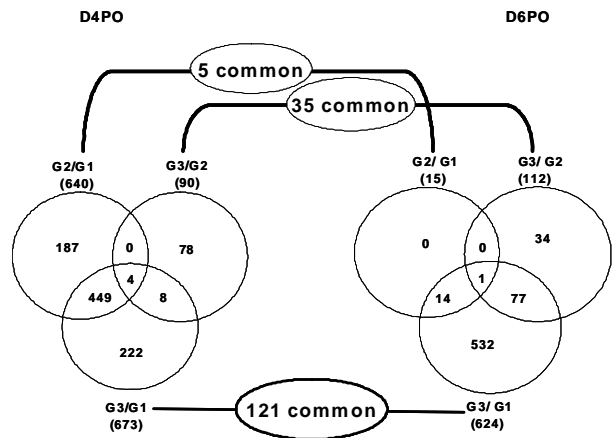

Fig. 1 : Characteristics of transcript expression in mid luteal phase endometrium of days 4 (D4PO) and 6 (D6PO) after ovulation under sufficient progesterone dominance in absence of embryo (group 1, G1), in presence of time synchronous preimplantation embryo, morula/early blastocyst on D4PO and blastocyst on D6PO (group 2, G2), and under progesterone starvation due to progesterone receptor antagonism by early luteal phase (day 2 after ovulation) mifepristone (2 mg/kg body weight, s.c.) in a fecund cycle with retrievable preimplantation embryo (group 3, G3). Venn diagrams show the quantitative characteristics and commonality among groups (G2: G1, G2/G1; G3: G2, G3/G2; G3: G1, G3/G1 and between days (D4PO and D6PO) for the transcripts that showed significant differential expression (more than 2-fold between groups) in cDNA based array analysis against ~1200 gene products.

(termed as *sufficient ecosystem*). It appears that the expressional-networks under progesterone as the prime mover bears considerable potential toward evolving small-world transcript networks depending on the nature and the course of high dimension attractor, namely embryo-derived signals, applied onto it.

In the real world, trajectories of a sustained, non-transient, dynamical system tend to cease by dissipation unless some driving force or an attractor is in operation.

Thus, an attractor typically provides a set of constraints to a system to make it evolve as a dynamical system (see reference 3 for details). Stage-synchronous pre-implantation embryo (PIE) behaves like a high dimension attractor, thus, it out-limits dissipative processes in progesterone dominated endometrium. The PIE mediated attractor properties converge onto the progesterone determined properties in the implantation stage endometrium in physiologically responsive manner towards endometrial receptivity and blastocyst implantation.

The example of differential regulation of mucin in implantation stage endometrium by sex steroid hormones and implanting blastocyst clarify the above-mentioned issue of prime mover action of progesterone and attractor function PIE (30, 31). It has been shown that human endometrial epithelial cells under progesterone dominance secrete mucin that protects the maternal endometrium from parasitic adhesion and invasion, while implanting blastocyst down-

regulates mucin to facilitate its implantation (31). This behaviour is species specifically selected depending on larger ecological and evolutionary coordinates (30), and also not mediated by single embryo-derived factor (32).

As explained in Fig. 2, small-world-ness emerges as the result of replacing a fraction (p) of links of a dimensional lattice interpolating between a regular lattice (p=0) and a random graph (p=1) (33, 34). Such networks are characterized by high degrees of clustering and short path lengths. It results in the emergence of a physiological process that appears to be both deterministic and vulnerable (35). The administration of a high affinity progesterone antagonist (like mifepristone having ten times more affinity to progesterone receptor than progesterone itself), thus, can inject significant noise into the expressional networks (Fig. 3B), making the coding process entropized and resulting in failure of blastocyst implantation (18, 19). The example of endometrial immunology
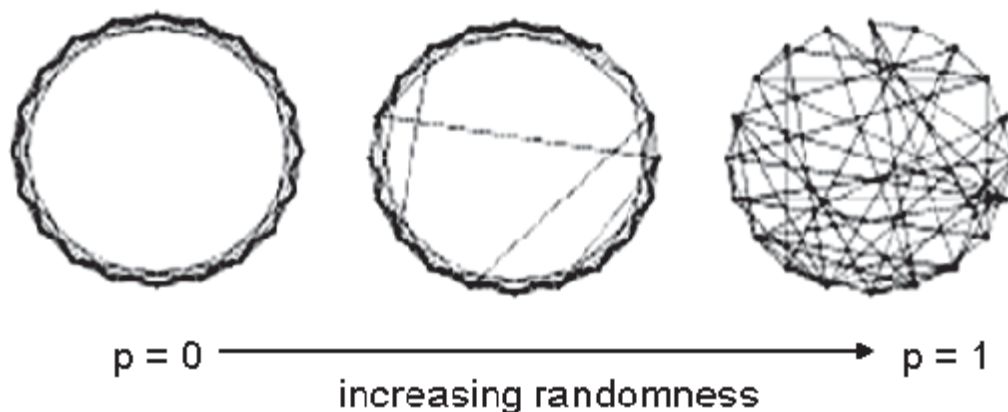

Fig. 2 : Two spectrums of networks. In many biological networks, clusters of nodes are assorted into tight clusters and maintain relatively short path links across the networks, giving rise to a small-world in the networks. The small-world-ness emerge as the result of replacement of a fraction (p) of links of a dimensional lattice interpolating between the two limiting cases, a regular lattice (p=0) and a random graph (p=1). Such networks are characterized by high degrees of clustering and short path lengths. See Almaas 2007 (26) for details.
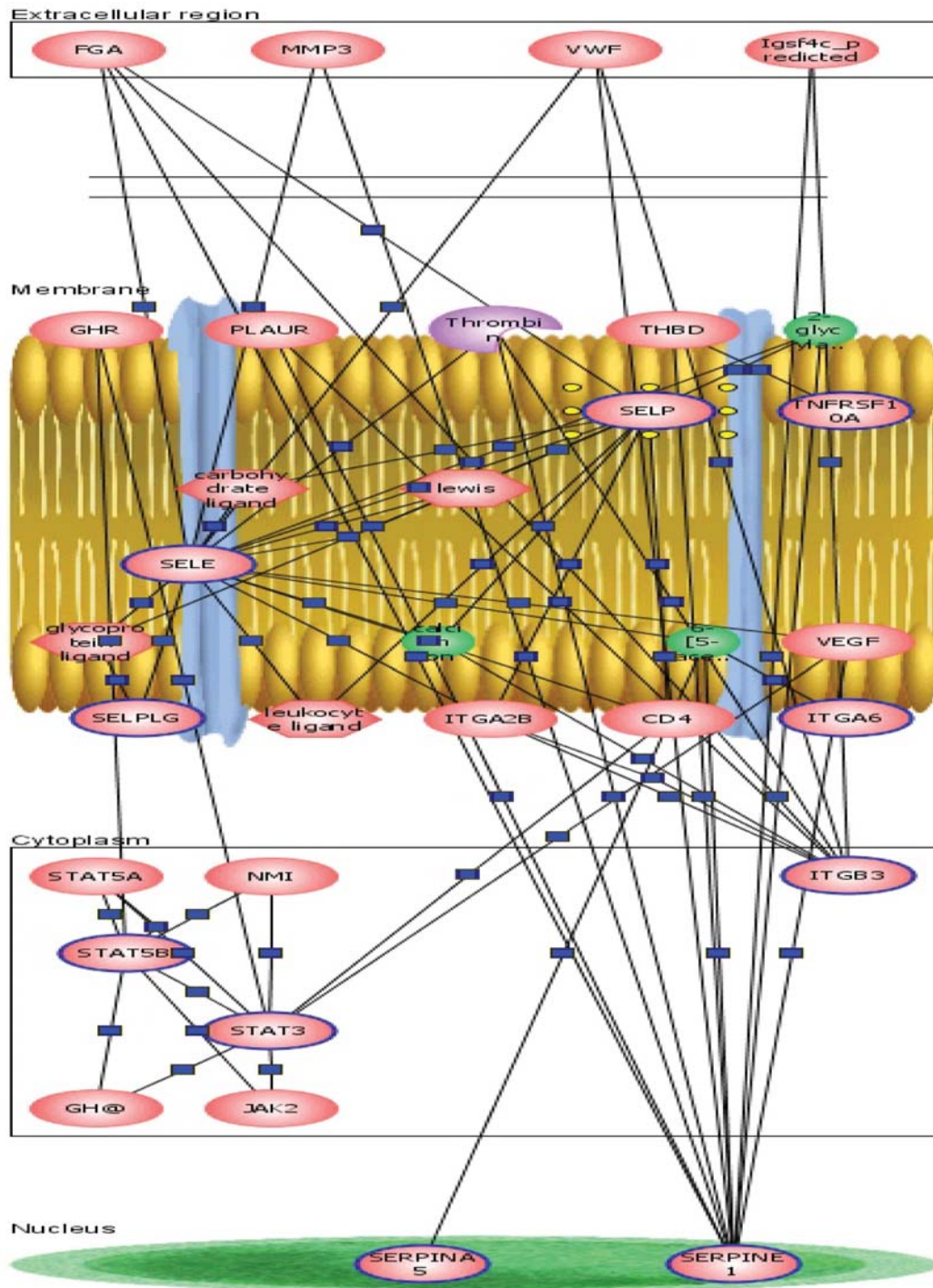
Fig. 3(A)

Fig. 3 : The small-world-ness of endometrial transcript networks in sufficient ecosystem (i.e., adequate progesterone and viable preimplantation stage embryo) on day 6 after ovulation (A), and its entropization by administration of low dose high affinity anti-progestin (mifepristone, 2 mg/kg body weight) (B) is seen. The probable physiological significance of observations as shown in (A) and (B) has been discussed in the essay.
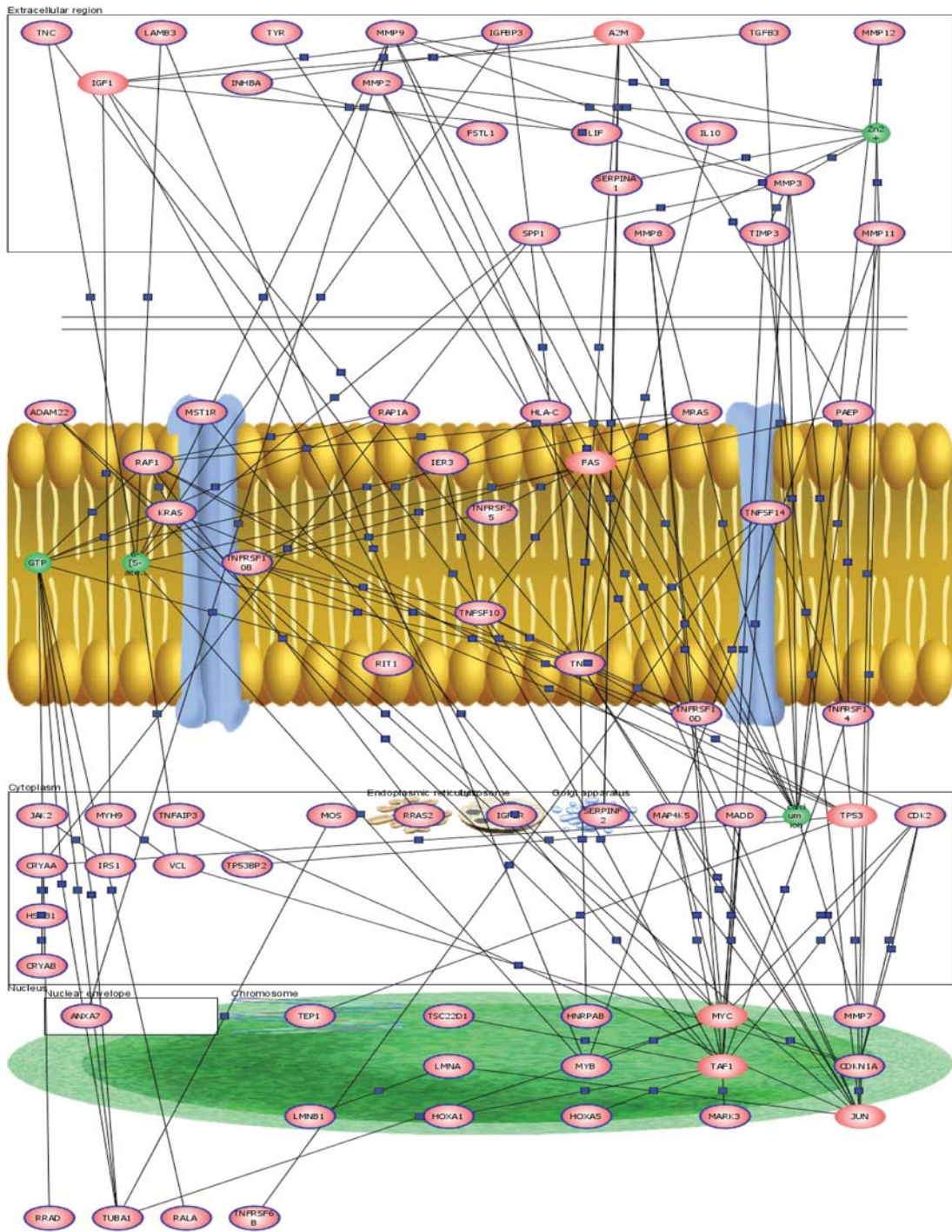
Fig. 3B.

with or without progesterone antagonism explains the issue of noise-effect on cellular signaling in implantation stage endometrium. While natural killer (NK) cells show high migratory ability into human implantation stage endometrium because of progesterone-dependent and possibly embryo-derived signals, administration of anti-progestin, mifepristone, however resulted in higher migration of macrophage and neutrophils with no change in NK cells in early pregnancy stage endometrium along with the termination of pregnancy (36–38).

In lieu of conclusion

The aim of the essay is not to draw any final conclusion. It is rather an attempt to examine blastocyst implantation in the primate as a process that can be explored from the view point of systems biology. We believe that there are two kinds of futurism in the endometrial transcriptomic networks application with specific reference to our approach and interpretation of endometrial receptivity.

Firstly, our proposed model should be further examined using high throughput systems biology approach especially to assess the veracity and biological tangibility of the assumptions and tools suggested. Secondly, the usefulness of the model may further be checked by introducing different grades of movers, attractors, as well as, noises in timed manner. For example, we have demonstrated that intra-uterine administration of oil and thread may provide embryo-analogous but distinguishable attractor function in the uterus of the rhesus monkey (39). Furthermore, local and

systemic application of progestin agonists and antagonists and other chemical and physical agents during early pregnancy may provide mover and noise functions of different qualities (36, 37, 40–42). It appears possible from the present model that high affinity anti-progestin may cause insufficiency in endometrial receptivity from increment in overall gain along with attenuation of signal-noise ratio despite cohorts of specific signals being accentuated (37, 42).

There exists a limitation in the proposed model approach. We have proposed in the present study that the complex transcriptomics in endometrium following interplay between embryo and endometrium under adequate hormonal milieu resulting in successful implantation process is associated with transformation of the expression networks to a small-world pattern with potential high determinism and vulnerability (20–22). However, the application of broad based systems biology approach is always limited in a low throughput study performed on selected number of gene products as employed in the present study design. Furthermore, our study employed PCR products of human genome retrieved from human genome project. Rhesus macaque genome project has made PCR products of monkey genes available in the open access research domain only very recently (43). Although there is good parsimony of 93% in genomic sequence between the rhesus monkey and the human, the orthology among genes of two species are discrete (44). Thus, the data obtained using rhesus monkey RNA and PCR products of 400 and 1200 genes from human genome bear a bias of false detection. We believe that, with the rhesus monkey as the non-

human primate model and with robust tools of systems biology at our disposal, the present model should be further explored using high throughput global gene expressional array technology for the rhesus monkey to investigate the systems biological basis of informational networks of endometrial transcriptomes in the process of endometrial receptivity for blastocyst implantation and that it shall yield future leads having high strategic significance in human reproduction.

Finally, it is notable that understanding of the key factors in endometrial receptivity has been a subject of intense study using non-primate and primate (human and non-human subjects) models for nearly six decades. While significant advancement has been made in this area, there have been limited attempts to systematically analyze and integrate the cohorts of factors in the context of the principal actors namely, embryo, endometrium and the hormonal milieu in an integrated manner using systems biology approach and robust tools like high throughput transcriptomics and proteomics, and computational modeling. The present article addresses this issue by developing transcriptomic networks of gene expressions of primate (macaque) endometrium in: (i) presence or absence of

embryo but with normal hormonal milieu, and (ii) presence of embryo but with and without progesterone starvation (caused by early luteal phase low dose mifepristone administration resulting in insufficiency in endometrial receptivity). Our study reveals that presence of a viable embryo introduces small-world type transcriptomic networks in the competent and adequately progesterone primed endometrium. This results in a physiological process to support blastocyst implantation. However, validation of the proposed principle of transcriptomic networks shall emerge from the study of high throughput endometrial transcript networks involved in the implantation process. We believe that the proposed systems biology approach to understand the endometrial physiology in the process of blastocyst implantation shall help to explain phenomena like delayed implantation, as well as, idiopathic failure of implantation in natural set-up and following IVF-ET.

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