

Role of Angiogenesis on Uterine Fibroids Therapy: review

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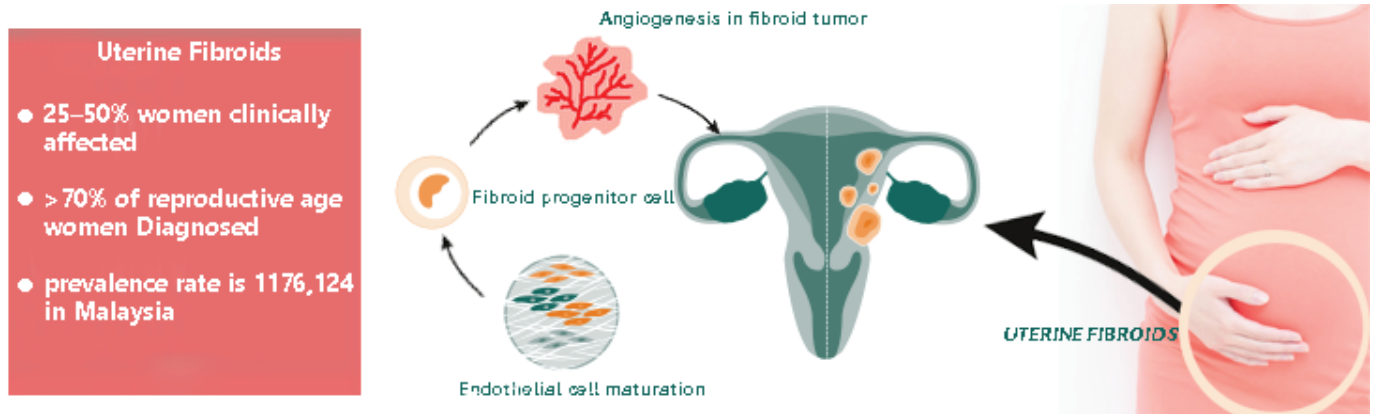
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Significance | A brief pathological understanding of the uterine fibroid (UF) and its study in animal is described in this paper. The development of an anti-angiogenic therapy is yet to be studied for the treatment of UF.

Graphical Abstract



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Role of Angiogenesis on Uterine Fibroids Therapy: review

Mahfoudh AL-Musali Mohammed Abdulghani

Abstract

Anti-angiogenic agents have recently been used in the treatment of cancer in combination with chemotherapy as angiogenesis is very well documented to be a powerful control point in tumour development. The growth of uterine fibroids was recently shown to be dependent on angiogenesis and different angiogenic factors involved in uterine fibroids are expressed in leiomyoma. Uterine fibroid (uterine leiomyomas) is one of the causes of infertility and is associated with recurrent miscarriages. Pathologically uterine fibroid is a benign tumours arising from the uterine myometrial compartment. Common treatments for fibroids include pharmacotherapy and non-pharmacotherapy treatments partly via anti-angiogenic mechanisms. In conclusion, direct anti-angiogenic agents may contribute to fibroid treatment. Unfortunately, there lacks good in vivo and in vitro models of uterine fibroid. Further research into developing experimental model to study uterine fibroid will aid in better understanding this disease.

Keywords: Angiogenic Factors, Uterine Fibroids, uterine leiomyomas

Abbreviations: VEGF, vascular endothelial growth factor

1. Introduction

The link between angiogenesis and tumours was first described in

Significance | A brief pathological understanding of the uterine fibroid (UF) and its study in animal is described in this paper. The development of an anti-angiogenic therapy is yet to be studied for the treatment of UF.

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1968 by Algire and Chalkley (Tonini et al., 2003). Currently, angiogenesis is very well-documented as a powerful control point in tumour development. Disordered structure in a tumour is due to an imbalance in angiogenic factors, like vascular endothelial growth factor (VEGF) and angiopoietins (Baish and Jain, 2000). Angiogenesis is the result of the coordination between induced angiogenic and anti-angiogenic factors (Makrilia et al., 2009). The growth, progression and metastasis of a tumour can be controlled by inhibiting the angiogenic process and this can be used for treatment of tumours as an alternative or additive therapy to conventional chemotherapy. In 2004, anti-angiogenic therapies in combination with chemotherapy for cancer began with the approval of bevacizumab by the US Food and Drug Administration (FDA) (Makrilia et al., 2009). Recently, angiogenesis was linked to uterine fibroids as a regulation of smooth muscle cell proliferation. The smooth muscle cell (SMC) proliferation is altered in fibroids compared with the adjacent myometrium. There is an exaggerated response to oestrogen, which promotes proliferation. Progesterone also appears to play a key role in stimulating SMC proliferation. A variety of growth factors (TGF- β , EGF, bFGF, IGFs, PDGF) have differential effects on fibroids compared with normal myometrium via a variety of mechanisms including alteration of receptor levels and signalling pathways. (Fleischer et al., 2008). Although uterine fibroids are considered benign tumours with reduced vascularization, abnormal vasculature of uterine fibroid was recently demonstrated. Different angiogenic factors involved in uterine fibroids are expressed in leiomyoma. These include epidermal growth factor (EGF), heparin-binding-EGF, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived

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growth factor (PDGF), transforming growth factor- β (TGF- β) and adrenomedullin. Common treatments for fibroids include pharmacotherapy and non-pharmacotherapy treatments partly via anti-angiogenic mechanisms. Direct anti-angiogenic agents may contribute to fibroid treatment (Tal and Segars, 2013).

There are three main approaches to treat fibroids including surgical, non-pharmacotherapy and pharmacotherapy. Firstly, the surgical treatment of uterine fibroid is considered the standard of care with full hysterectomy being the definitive option, representing a “cure” for the disease. However, traditional myomectomy via laparotomy entails considerable morbidity and is commonly associated with adhesion formation, which may lead to loss fertility and, on occasion, may even result in bowel obstruction. Secondly, uterine artery embolization (UAE) as a non-pharmacotherapy commonly used in the treatment of uterine fibroid without surgery, leads to blood flow obstruction to the fibroid. In pharmacotherapy, several groups of drug have been shown to manage symptoms before surgery for uterine fibroids. Gonadotropin-releasing hormone (GnRH) agonists are commonly used, Selective Estrogen Receptor modulators, progesterone receptor antagonist and Selective Progesterone Receptor Modulators (SPRMs).

Uterine fibroids are known as uterine leiomyomas arising from the uterine myometrial compartment. They are the most common tumours of the female reproductive tract (Walker et al., 2003). Uterine fibroid can be pathologically classified as solid tumour diseases as they have a relatively low mitotic index and retain their smooth muscle phenotype. Unfortunately, they can cause significant morbidity and even mortality. Uterine fibroids clinically affect 25–50% of all women (Walker et al., 2003). They occur with a remarkable frequency in more than 70% of reproductive-aged women (Cramer and Patel, 1990). Symptomatic fibroids can be associated with several problems including menorrhagia, pelvic pressure, pelvic pain, recurrent miscarriage and infertility. In the United States, due to the previous clinical problems, uterine fibroids are responsible for about one-third of all hospital admissions for gynaecological services, and approximately 175,000–370,000 hysterectomies performed annually (Hoffman et al., 2004). In Malaysia, uterine fibroid prevalence rate was estimated as 1,176,124 based on population of 23,522,482 according to Right Diagnosis for health grades.

Relatively little is known of the biology, aetiology and pathophysiology of uterine fibroid (Hoffman et al., 2004) and their underlying causes are poorly understood. Sex steroids hormones and their receptors, glutamate, angiogenic factors, apoptosis factors and some genes showed upregulation and downregulation. Oestrogen receptors (ERs) and progesterone receptors (PRs) were also shown to be expressed higher than in normal myometrium. Screening of uterine fibroid and myometrial tissues

with DNA arrays revealed numerous genes upregulated in leiomyomata including glutamate receptor 2 (GluR2), nuclear receptor peroxisome proliferator-activated receptors (PPARs) and retinoid X receptor (RXR) proteins. GluR2, a subunit of a ligand-gated cation channel, is upregulated in fibroids relative to the myometrium by 15- to 30-fold at the protein and mRNA level and is localized on endothelial cells. GluR2 pre-mRNA in fibroids and myometrial tissues is nearly 100% edited at the Q/R site, indicative of low Ca^{2+} permeability of the ion channels. In spontaneous fibroids in women or fibroids induced in the guinea pig model, increased production of estradiol and all trans retinoic acid with up-regulation of nuclear receptor PPAR and RXR proteins are likely to synergize to support tumor growth (Tsibris et al., 2003). In a study of genome-wide miRNA expression patterns in uterine fibroids and myometrium using Solexa high-throughput sequencing, a study found more than 55 miRNAs which were differentially expressed. The top five significantly de-regulated miRNAs in uterine fibroids that was found in their libraries were miR-363, miR-490, miR-137, miR-217 and miR-4792. It was also observed “isomiRs” with higher copy number than referenced mature miRNA specific for the leiomyoma libraries, which have a potential role in tumorigenesis (Georgieva et al., 2012). In study for quantification of transcript expression levels in uterine fibroids relative to normal myometrium using Model-based expression analysis revealed that of the 22,500 transcripts represented on the arrays, 226 genes were found to be dysregulated by a >1.5-fold change between leiomyoma and normal myometrium. Moreover, in this study, the authors identified many dysregulated apoptosis-related genes. Of particular interest was TRAIL (Tumor necrosis factor-related apoptosis-inducing ligand) and Ask1. Numerous differentially expressed proliferation genes, including TGF β 1, PDGFC, and two dual specificity phosphatases were also identified. These genes may play a significant role in the development of leiomyomas from normal uterine tissue. The deregulation of apoptotic and proliferative processes has been hypothesized to be key to fibroid development (Hoffman et al., 2004).

Experimental models of uterine fibroid have been documented in a literature review include a range of in vitro to ex vivo and in vivo using enzyme based, cell-based, rodent animal and non-rodent animals. In vitro experimental models, cell-based (Leiomyoma tumor-cell lines) has been used which are derived from Eker rats called ELT cells line or from patient’s uterine fibroid samples. Several outcomes came from this study, including information of antiestrogenic activity, expression of estrogenic receptors, apoptotic activity of tested compounds, anti-angiogenesis activity, protein expression, and gene expression.

ELT cells line (Eker leiomyoma tumour) are one of the numerous cell lines that have been developed from Eker rat uterine fibroids (Howe et al., 1995). These cell lines have been given the designa-

tion ELT (for Eker leiomyoma tumor-derived) and five such lines have been established to date. All five cell lines (ELT-3; ELT-4; ELT-6; ELT-9; ELT-10) are positive for the expression of smooth muscle α and β actins and desmin by northern analysis and immunocytochemistry, but ELT-3 is also positive for expression of estrogenic and are tumorigenic in nude mice. The anti-angiogenic activity of metformin was observed in ELT-3 cells by suppressing the expression of VEGF via the mTORC1/HIF-1 α pathway (Tadakawa et al., 2015). The Eker rat model and tumour-derived ELT cell lines have been used extensively to identify therapeutic strategies for uterine fibroids and to investigate the aetiology of this disease.

1.2 In vitro studies have been performed using immortalized cultures of patient-matched leiomyoma and myometrium. Patient(s)

Women undergoing hysterectomy for symptomatic leiomyomas. Intervention(s): Immortalized cell cultures, quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR), cytoimmunofluorescence, and Western blot analysis. Main outcome measure(s): Morphologic features; expression of COL1A1, versican, fibronectin, dermatopontin, and transforming growth factor β 3 (TGF- β 3); and integrin-mediated 3D structural formation. This model system allows for assessment of the mechanism of aberrant ECM formation as well as the effectiveness of various potential therapies. (Malik and Catherino, 2012; Joseph et al., 2010). Some of the upregulated genes were for enolase, sex-steroid receptor cofactors, proteins in insulin pathways and CD24 and CD97. Some of the down-regulated genes were dermatopontin, glucocorticoid receptor and CD4. (Verginadis et al., 2011).

1.3 Ex vivo Experimental modules

Estrogen will be administered to animals, then the concentration of estrogen receptors (ERs) in myometrium will be determined. In general, both ERa and ERb are present in higher levels in leiomyoma tissue compared with myometrium. 45 ERb appears to be ubiquitous to all cell types within myometrium, whereas ERa has a variable expression in different cell types. This finding indicates that ERb is more relevant to the development and growth of leiomyomas. Another finding of this study implies that oestrogen acts upon growth factors, cytokines, histamine and heparin to promote cell proliferation (Fleischer et al., 2008).

1.4 In vivo Experimental modules

Five animal models have been found in the literature review which include inoculated nude mice with ELT-3 cells (Vaezy et al., 2000), Eker Rat Model (Yeung et al., 1995), Monosodium glutamate (MSG) treated rats Obochi et al., 2009), Dehydroepiandrosterone (DHEA) treated rats (Zhang et al., 2013) and guinea pig models of estrogen-induced uterine fibroids (Porter et al., 1995).

1.4.1 The first animal model, inoculated nude mice with ELT-3 cells as model for fibroid

The method by (Vaezy et al., 2000) is as follows, each nude mice was inoculated with 3 to 5 \times 10⁶ ELT-3 cells at a single suprascapular site between the ages of 8 and 10 weeks. Once a tumour was detected, transcutaneous caliper measurements were used to measure the long and short dimensions of the tumour. Tumour volume was calculated on the assumption of an ellipsoidal volume, which was observed to be the predominant shape of the tumours. The tumour volume in milliliters was calculated according to the following formula: $V = 4/3\pi ab^2$ (Where V represents volume and a & b represent the semiaxial dimensions (half of the long and short dimensions, respectively) of the tumour in centimetres. Animals in which a tumour developed were randomly assigned to one of three groups: no treatment (positive control), different groups of treatment, or sham treatment.

1.4.2 Eker Rat as second model

The Eker rat is the only animal model that spontaneously develops uterine fibroids (Everitt et al., 1995). By 1–1.5 years of age, female carriers in this line develop easily observable reproductive tract fibroids that share many characteristics with human fibroids. Eker rats carry a defect in the tuberous sclerosis gene 2 (Tsc-2) tumour suppressor gene and female Eker rats develop uterine fibroids with a high frequency. The uterus is a site of high expression of tuberlin, the product of the Tsc-2 gene, and tumours that arise in heterozygous animals show loss of tuberlin function. At the DNA level, loss of heterozygosity (LOH) at the Tsc-2 locus is commonly observed in these tumours, with loss of tuberlin function occurring by several mechanisms, including loss of chromosome 10 on which the wild-type Tsc-2 gene is located (either monosomy or chromosome nondisjunction with retention of two copies of chromosome 10 containing the mutant allele), gene silencing, and point mutations (Yeung et al., 1995). However this animal model has limitation, Eker rat is the genetic experimental model for uterine fibroids which accurately describes cases where patients have “genetic” syndromes that include fibroid development, such as hereditary fibroids, but is not suitable for modelling uterine fibroids that are independent of genetics such as sex hormone levels and environmental factors (Walker et al., 2003). The third animal model is Monosodium glutamate (MSG)-induced fibroids in rats by increased serum estrogen and cholesterol: Different dosages of MSG have been used to induce fibroids in rats. The dosages which were found include 100 mg/kg (Obochi et al., 2009), 200 mg/kg (Ogunlabi et al., 2014) to 800 mg/kg (Koffuor et al., 2013) of body weight of the animals. The duration of treatment is between to 2 months. Several parameters were measured such total serum sex hormones, cholesterol and serum total protein, uterus weight-to-body weight ratios. The fourth animal model, dehydroepiandrosterone (DHEA)-induced

hyperfibrosis in uterine of rats: PCOS rat models induced by daily subcutaneous administration of DHEA with dose of 60/kg/2 ml of sesame oil to 3 week-old rats for 35 consecutive days exhibit significantly higher levels of fibrosis (collagen IV) in uterine tissues. The uteri showed a thinner layer of endometrium, a thicker layer of myometrium and a higher level of collagen accumulation, all of which suggested a higher level of fibrosis in rats with PCOS. TGF- β and CTGF, two key pro-fibrotic factors, showed upregulated expression in uteri of these rats. Similarly, mRNA levels of fibronectin and angiotensin-II showed increased expression (Zhang et al., 2013). The fifth animal model, guinea pig model of estrogen-induced uterine fibroids: Guinea pig aged ranging from young to old with average age from 4-6 months to 2-2.5 years old received two E2 in the subcutaneous space of the intrascapular area 7-10 days after a bilateral oophorectomy or sham operation for three months. The parameters can be evaluated serum sex hormones and uterus tissue (Porter et al., 1995).

2. Discussion

Recent study about anti-angiogenic agents in combination with standard chemotherapy for the treatment of cancer indicated that direct anti-angiogenic agents may contribute to uterine fibroid treatment (Ferrara and Kerbel, 2005). The current pharmacotherapy for uterine fibroids showed many limitation because of low specificity which leads to serious side effects. The GnRH-agonists effectively shrink uterine fibroid volume due to the hypo-estrogenic state induced (Andreyko et al., 1987; Adamson, 1992) and it can cause a significant loss of bone mineral density due to extended mechanism of action (Dawood et al., 1989). Because of this side effects, treatments are limited to six months in length. Moreover, cessation of therapy results in regrowth of uterine fibroids, often rapidly (Friedman et al., 1990).

The biological studies on uterine fibroids have revealed that the abnormalities of angiogenic and anti-angiogenic factors are responsible for the growth of uterine fibroids. The estrogen receptors (ERs), progesterone receptors (PRs), glutamate receptor 2 (GluR2) nuclear receptor peroxisome proliferator-activated receptors (PPARs) and retinoid X receptor (RXR) are involved in uterine fibroid through either direct or indirect effects by interfering in the balance between angiogenic and anti-angiogenic factors. A gene study of uterine fibroid indicated that 226 genes were found to be dysregulated by a >1.5-fold change between uterine fibroid and normal myometrium (Hoffman et al., 2004).

The biology of uterine fibroid, aetiology, pathophysiology, and the underlying causes are not clearly understood because of the limitations of experimental models used to study uterine fibroids. Experimental models of uterine fibroid have been documented in a literature review that include a range of in vitro and to ex vivo as well as in vivo enzyme based, cell-based, rodent animal and

non-rodent animals. In vitro experimental models, cell based (Leiomyoma tumour-cell lines) has been used which are derived from Eker rats called ELT cells line or from patient's uterine fibroid samples. Several discoveries were made using these models which include antiestrogenic activity, expression of estrogenic receptors, apoptotic activity of tested compounds, anti-angiogenics activity, protein expression, and gene expression in these uterine fibroids. The in vivo experimental models used were five different animal models that include inoculated nude mice with ELT-3 cells (Vaezy et al., 2000), the Eker Rat Model (Yeung et al., 1995), rats treated with monosodium glutamate (MSG) (Obochi et al., 2009), rats treated with dehydroepiandrosterone (DHEA) (Zhang et al., 2013) and a guinea pig model of estrogen-induced uterine fibroids (Porter et al., 1995).

3. Conclusion

In conclusion, direct anti-angiogenic agents may contribute to fibroid treatment. However, there still exists a lack of good in vivo and in vitro models of uterine fibroid. Further research in order to develop experimental models will help drive research forward to study the anti-angiogenic effects of various treatments on uterine fibroid.

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Author Contribution

Mahfoudh AL-Musali M. A. made substantial contributions to the conception design of the manuscript, and review of the literature.

Competing financial interests

The author(s) declare no competing financial interests.

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