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Review

Delineating the molecular mechanisms of tamoxifen's oncolytic actions in estrogen receptor-negative cancers

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ABSTRACT

Since its clinical inception, tamoxifen (TAM) has proved to be a powerful tool in treating estrogen receptor-positive breast cancers while exhibiting manageable side effects. Although TAM was synthesized as an estrogen receptor antagonist, reports have found that a significant fraction of women with estrogen receptor-negative cancers have benefitted from TAM treatment, suggesting the possibility of an alternate anti-cancer mechanism. In this paper, we present a review of recent and past literature in an attempt to clarify how TAM inhibits cell proliferation and induces apoptosis in cells lacking the estrogen receptor. Our analysis indicates that micromolar concentrations of TAM selectively elevate intracellular calcium concentrations in malignant cells, possibly by inversely agonizing cannabinoid receptors, producing considerable mitochondrial distress followed by the rapid production of reactive oxygen species. In response, cytoplasmic proteins such as JNK1 are activated, which mediates the activation of caspase-8. Fyn kinase auto phosphorylates in response to increased reactive oxygen species and directs the ubiquitin ligase c-Cbl to tag growth factor receptors for ubiquitination, potentially abrogating constitutively active survival pathways that are hallmarks of cancer progression. We attempt to differentiate the effect that TAM has on purified Protein Kinase C (PKC) compared to that in an intact cell, suggesting that low micromolar concentrations of TAM indirectly inhibit PKC by inducing EGFR destruction and high micromolar concentrations of TAM inhibits PKC through a direct binding mechanism.

Contents

1. Introduction

Tamoxifen (TAM) was originally synthesized as a competitive estrogen receptor (ER) antagonist with nanomolar binding affinity

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(O'Brian et al., 1985, 1990). After its clinical translation in the 1970s, it quickly became a widespread and effective first-line treatment for ER+ breast cancers while exhibiting minimal side effects. TAM's primary mechanism of action was supported by the fact that 17ß-estradiol (E2) dose-dependently abrogated TAM's oncolytic effects and that TAM antagonizes the oncogenic effects of estrogen (Charlier et al., 1995).

TAM has been prescribed as a chemo-preventive therapy for women with a high risk of metastatic ER+ breast cancer. Results from randomized clinical trials demonstrate a 35% decrease in the occurrence of contralateral breast cancers in women taking TAM compared to those taking placebo (Nayfield et al., 1991). Clinical studies using TAM demonstrated that 30% of women with ER- breast cancers responded to TAM treatment (Tormey et al., 1976) as well as a significant subset of patients with recurrent malignant glioma (Couldwell et al., 1996). TAM in combination with sulindac produced partial or complete responses in a majority of patients with desmoid tumors (Hansmann et al., 2004) while TAM and gemcitabine provided clinical benefit to 59% of patients with advanced pancreatic carcinoma (Tomao et al., 2002). In accordance with these clinical findings, Reddel et al. (1985) reported that TAM exhibited in vitro oncolytic activity in several ER- cancerous cell lines, an effect that was not reversible by simultaneous high-dose E2 administration. In addition, TAM's ability to induce apoptosis in MCF-7 cells, a widely used ER+ breast cancer cell line, was observed using micromolar drug concentrations, suggesting TAM might display alternative mechanisms of action at higher doses (Yan et al., 2011).

Since the discovery that TAM could inhibit proliferation and induce apoptosis in ER- cancers (Reddel, 1985), a myriad of reports have attempted to elucidate the ER-independent anti-cancer mechanisms of TAM. Initial reports suggested that TAM induced apoptosis in ER- cancers by increasing cellular oxidative status (Gundimeda et al., 1996) (Table 1), inhibiting Protein Kinase C (PKC) (O'Brian et al., 1990) and as a consequence, inhibiting DNA synthesis and proliferation in malignant gliomas (Pollack et al., 1990). Furthermore, TAM has been shown to elevate cytosolic (Kim et al. 1999) and mitochondrial calcium levels (Nazarewicz et al., 2007), modulate c-Jun NH_2-terminal kinase (JNK) 1 activity (Mandlekar et al., 2000a) and induce Transforming Growth Factor Beta (TGF-ß) production and secretion (Perry et al., 1995). More recent work has shown that TAM induces the degradation and/or inactivation of proteins that are vital to proliferation, chemotherapeutic drug resistance, and metastasis of tumor cells to neighboring tissues (Scandlyn et al., 2008) (Fig. 1).

In the present work, we review the pre-clinical and mechanistic work over the past forty years that have attempted to elucidate the multiple mechanisms by which TAM induces cell growth inhibition and apoptosis in a number of cancers that lack the ER. We place specific emphasis on TAM's impact on mitochondrial physiology, the cellular consequences of its ability to raise intracellular oxidative status, its effects on the proteasome system and attempt to resolve its differential effects on PKC activity while reviewing some additional mechanisms of action.

Table 1
Effect of Tamoxifen on different ER-negative cancers.

Cancer	Effect *in vitro*	Reactive Oxygen Species Production	Cellular targets	Apoptosis	Effect *in vivo*	Reference
Breast Cancer	Cell viability↓	+	p-Fyn Kinase↑ p-C-Cbl↑ EGFR↓	+	Tumor growth↓	Chen et al. (2013)
Breast Cancer	Cell viability ↓	NT	EGFR↓ Akt↓ p-Akt↓ NfKB↓	+	Tumor growth↓	Scandlyn et al. (2008)
Breast Cancer	Cell viability ↓	+	Caspase-3, -6, -8, -9 ↑ JNK1 ↑	+	NT	Mandlekar et al. (2000)
Breast Cancer	Cell viability ↓	+	PKC activity ↓	+	NT	Gundimeda et al. (1996)
Cholangiocarcinoma	Cell viability↓	NT	c-Flip↓ p-Akt↓ Caspase-3, -8, -9, -10 ↑	+	Tumor growth↓	Pawar et al. (2009)
Glioblastoma	Cell viability ↓	NT	p-PKC↓	+	NT	Balça-Silva et al. (2014)
Glioma	Cell viability↓ Cell cycle progression ↓	NT	caspase-3 activity↑ PKC-ι activity ↓ Bcl-2 ↑ Cdk7 activity↓	+	NT	Yang et al. (2015)
Glioma	Cell viability↓	NT	PKC activity↓ Insulin-like growth factor II ↓	+	NT	Ramachandran et al. (2004)
Glioma	Cell viability↓	NT	JNK1↑ p-c-Jun↑ FasL↑	+	NT	Moodbidri and Shirsat (2005)
Leukemia	Cell viability↓	+	NOS activity↑ NOS expression ↑	+	NT	Maccarrone et al. (1998)
Liver Cancer	Cell viability↓	NT	Calcium influx↑	+	NT	Kim et al. (1999)
Liver Cancer	Cell viability↓	+	Calcium influx↑ NAD(P)H Oxidase activity ↑	+	NT	Lee et al. (2000)
Liver Cancer	Cell viability↓	+	PKC Activity↓ Telomerase Activity↓	+	NT	Brandt et al. (2005)
Murine Melanoma	Invasion↓ Migration ↓	NT	MMPs↓ p-ERK 1/2↓ p-AKT↓ p-PKCα↓ p-PKCδ↓	NT	Lung metastasis↓ Primary tumor metastasis ↓	Matsuoka et al. (2009)
Pancreatic Cancer	Cell viability↓	NT	p-PKCα↓	+	NT	Xie et al. (2014)
Prostate Cancer	Cell viability↓	NT	PKC activity↓ p21↑ RB protein activity↑ G1/S phase cell cycle arrest↑	+	NT	Rohlff et al. (1998)
Thyroid Cancer	Cell growth↓ Invasion↓	NT	PKC↓	+	Tumor growth↓	Hoelting et al. (1995)

NT- Not tested. ↑ Increase. ↓ Decrease.