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Review

Taxol synthesis

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Being a complex diterpenoid, the potent anticancer drug, Taxol, requires complicated steps for its biosynthesis. In the present article, recent advances on Taxol biosynthesis pathway are reviewed, including many recently reported genes that regulate Taxol biosynthesis. To meet the urgent need of clinic and scientific research, besides Taxus supply, other approaches to obtain Taxol have also been discussed here.

Key words: biosynthesis pathway, cell culture, endophytic fungi, Taxol, Taxus.

INTRODUCTION

Taxol (*paclitaxel*) is one of natural diterpenoid alkaloids firstly isolated from the bark of the yew (*Taxus brevifolia*) (Figure 1) (Wani et al., 1971). Because it can kill tumor cells by enhancing the assembly of microtubules and inhibiting their depolymerisation (Schiff et al., 1979), Taxol has been well established and approved by FDA (the Food and Drug Administration) as a very important effective chemotherapeutic agent against a wide range of tumors since 1992 (Kohler and Goldspiel, 1994). However, the supply of Taxol has been limited since the discovery of this natural product, and, with increasing applications in chemotherapy, the availability and cost of the drug will remain an important issue (Kwon et al., 1998).

Until now, all Taxol used in cancer chemotherapy and scientific research is isolated from yew tree or semi-synthesized from its precursors such as baccatin III and 10-deacetylbaccatin III which are all isolated from this natural plant (Denis et al., 1988). However, this natural resource is being threatened day by day due to the destructive collection of Taxus bark for Taxol. In order to protect Taxus in the world and lighten the pressure of Taxol sourcing, other approaches to obtain Taxol have been under investigation and some progresses have been made.

Besides semi-synthesis and isolation from plant, there are several other possible routes to industrialize Taxol production: tissue or cell culture (Christen et al., 1989; Hu et al., 2003), total chemical synthesis (Holton et al., 1994; Nicolaou et al., 1994; Morihira et al., 1998), fungal fermentation (Stierle et al., 1993; Strobel et al., 1996; Li et al., 1996; Wang et al., 2000) and a potential way of engineering for Taxol production.

In this review, we focus on describing the biosynthetic pathway of Taxol in Taxus, including many recently reported certain genes that regulate Taxol biosynthesis. We also discuss other approaches to obtaining Taxol and their advantages and disadvantages.

TAXOL FROM TAXUS

It is well known that Taxol was first isolated from yew tree and this natural plant is the main resource for Taxol production up till now. Also, the study of Taxol biosynthetic pathway was first carried out in *Taxus* and the biosynthetic mechanism of this complex diterpenoid has been basically elucidated.

Taxus

Taxus (yew) is a slow-growing evergreen shrub or small tree. There are altogether eleven Taxus species in the

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Table 1. The cloned genes involved in Taxol biosynthesis pathway in Taxus.

Enzyme	cDNA corresponding to the enzyme			Reference
	GenBank Accession No.	CDS (bp)	Enzyme (kDa)	
Taxadiene synthase	AY364469	2,586	98.3	Wildung et al., 1996
GGPPS	AF081514	1,182	42.6	Hefner et al., 1998
TAT	AF190130	1,317	49	Walker et al., 2000a
TBT	AF297618	1,320	50	Walker et al., 2000b
DBAT	AF193765	1,320	49	Walker et al., 2000c
Taxane 10-β hydroxylase	AF318211	1,494	56.7	Schoendorf et al., 2001
Taxane 13-α hydroxylase	AY056019	1,458	54.7	Jennewein et al., 2001
BAPT	AY082804	1,335	50	Walker et al., 2002a
DBTNBT	AF466397	1,323	49	Walker et al., 2002b
Taxane 2-α hydroxylase	AY518383	1,488	55	Chau et al., 2004a
Taxane 7-β hydroxylase	AY307951	1,503	56.3	Chau et al., 2004b
Taxane 5-α hydroxylase	AY289209	1,509	56.8	Jennewein et al., 2004
PAM	AY582743	2,094	76.5	Walker et al., 2004

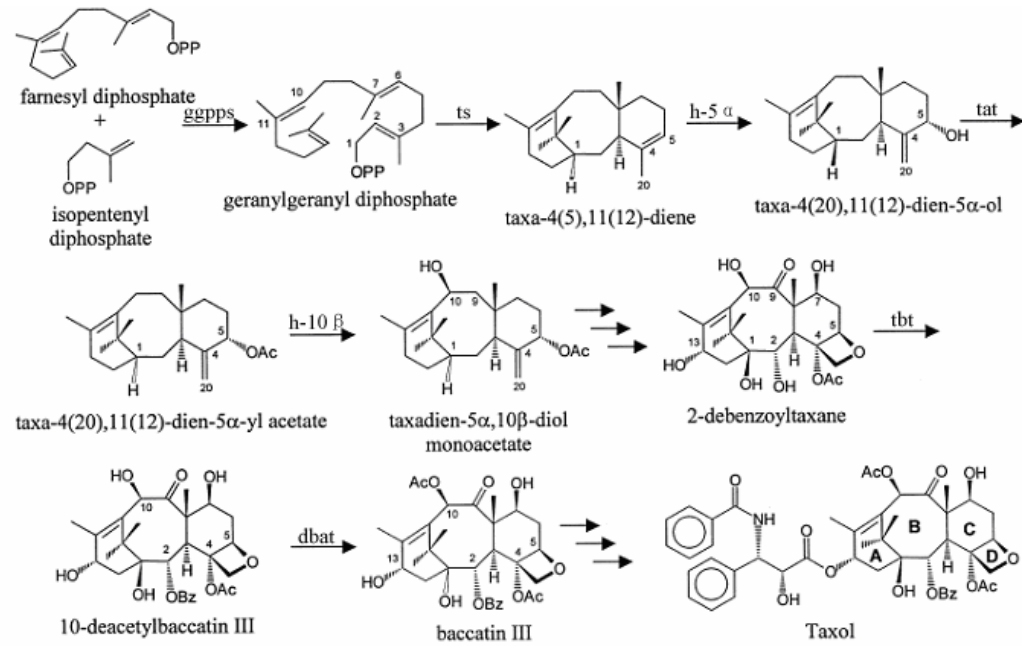

Figure 2. Taxol biosynthetic pathway. ggpps: geranylgeranyl diphosphate synthase; ts: taxadiene synthase; h-5α: cytochrome P450 taxadiene 5α-hydroxylase; tat: taxa-4(20), 11(12)-dien-5a-ol-O-acetyltransferase; h-10β: cytochrome P450 taxane 10β-hydroxylase; tbt: taxane 2a-O-benzoyltransferase; dbat: 10-deacetyl baccatin III-10-O-acetyltransferase. Multiple arrows indicate several as yet undefined steps.

world, sporadically distributed throughout northern temperate zones with an exception of AustroTaxus spicata located on the Southern hemisphere. The foliage, bark and seeds, but not the fleshy red aril, of Taxus contain a mixture of alkaloids, diterpenes, ligans, tannin and resin, making it extremely toxic. Since the discovery of Taxol, Taxus has attracted considerable attention. Among different Taxus species and different tissues of the tree, there is a variable Taxol production ranging from zero to 0.069% (Castor and Theodore, 1993; Guy et al., 2002).

Taxol biosynthesis in Taxus

Great progress has been made in the biosynthetic mechanism of Taxol in Taxus due to dozens of researchers' fundamental work, especially in the past ten years. Except for a few undefined steps, the Taxol biosynthetic pathway has been elucidated (Figure 2) and many genes encoding certain enzymes, which regulate Taxol biosynthesis pathway, have been cloned and characterized (Table 1).

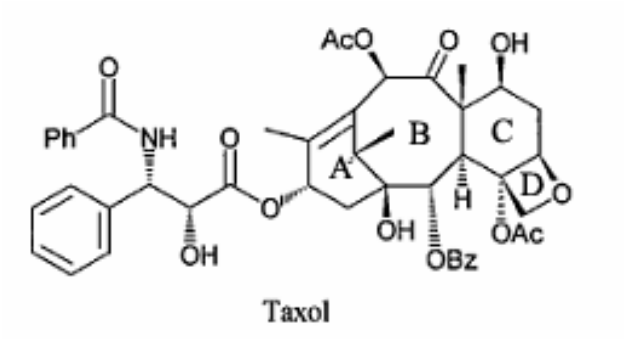

Figure 1. The chemical structure of Taxol with a molecular formula of $C_{47}H_{51}O_{14}N$ and a molecular weight of 853.92.

The Taxol biosynthetic pathway is considered to require 19 enzymatic steps from the universal diterpenoid precursor geranylgeranyl diphosphate (Hezari and Croteau, 1997) which is cyclized, in the committed step, to taxa-4(5), 11(12)-diene. This parental olefin is then functionalized by a series of eight cytochrome P450-mediated oxygenations, three CoA-dependent acylations, and several other transformations en route to baccatin III, to which the side chain at C13 is appended to afford final product Taxol (Figure 2). As to the diterpenoid precursor, geranylgeranyl diphosphate, previous biochemical studies have demonstrated that it can be derived from deoxyxylulose-5-phosphate pathway and the more common mevalonate pathway like those in the model plant of Arabidopsis thaliana (Srinivasan et al., 1996; Palazón et al., 2003; Wang et al., 2003; Laule et al., 2003).

In recent years, our research group has also done much underlying work on Taxol biosynthetic mechanism, and a few genes encoding certain enzymes, which catalyze the Taxol biosynthesis reactions, have been cloned and characterized (Kai et al., 2004; Liao et al., 2004).

Taxus supply

As stated above, due to the relative scarcity of Taxus trees, their slow growth, as well as the low content of Taxol in the trees, the supply of Taxol sustained by isolation from the original plant source is very limited. Nowadays, seedling culture and forestation have been widely considered as the most feasible methods to obtain Taxol and its chemical semi-synthetic precursors. At the same time, a hybrid Taxus species, Taxus media with needles containing high content of Taxol (Castor and Thedore, 1993) provides a good choice for commercial production of Taxol.

TAXOL FROM CELL CULTURE

Taxus cell culture has been considered as another promising means to produce Taxol and has been extensively researched. Since Christen et al. (1989) reported the first production of Taxol by Taxus cell cultures, which was later patented in 1991 (U.S. Patent No. 5,019,504), progress has been made in increasing Taxol yield in culture by feeding precursors (Chen et al., 1998; Fett-Neto et al., 1994; Furmanowa et al., 2000) and sugars (Chen et al., 1998; Choi et al., 2000; Ketchum and Gibson 1996), or using elicitors such as methyl jasmonate (Mirjalili and Linden 1996; Furmanowa et al., 1997; Ketchum et al., 1999; Yukimune et al., 1999), fungal cultures (Chen et al., 1999), vanadyl sulfate and chitosan (Cusido´ et al., 1999; Furmanowa et al., 2000). All of these studies show an enhanced content of Taxol by differently treated Taxus cell cultures compared with control. Among them, the highest yield of Taxol obtained in cell cultures is approximately 0.5% of dry weight by Yukimune et al. (1999) by adding an elicitor, methyl jasmonate. Factors influencing stability and recovery of paclitaxel from suspension cultures and the media have been studied in detail by Nguyen et al. (2001).

So far, although many Taxus species have been explored for production of Taxol using plant cell cultures and gained considerable success, it is still limited for large-scale commercial use because of the low and unstable product yield, as well as high production cost. Therefore, to meet the commercial need of Taxol, high Taxol- yielding and fast growing cell lines are needed, and the production cost should be reduced. This may be accomplished using cell selection in combination with medium optimization, elicitation and optimization of extraction process.

TOTAL SYNTHESIS OF TAXOL

The structural elements of Taxol, in addition to the main skeleton: A, B and C rings, include the oxetane ring (D-ring), the N-benzoyphenylisoserine side chain appended to C13 of the A-ring, and the benzoate group at C2 of the B-ring (Figure 1). In 1994, two research groups (Holton et al., 1994; Nicolaou et al., 1994) announced their exciting reports that total chemical synthesis of Taxol had been achieved with a low production rate of 2.7% and 0.07%, respectively. The route devised by the Nicolaou group employed a convergent synthetic plan involving the construction of the A and C rings separately and then coupling the two molecules together using a Shapiro and a McMurry coupling to form the B ring. Further reactions were then carried out to produce the final product Taxol. Holton and coworkers took a different approach to that used by Nicolaou choosing (-)-borneol as their starting material, which they converted to an unsaturated ketone over a total of 13 synthetic steps. Two years later, Danishefsky et al. (1996) devised the third route that required fewer steps than the Holton or Nicolaou routes. The method involved starting with the Wieland-Miescher ketone, which was then converted to a complex enol

triflate containing an olefin on the C-ring that allows for the development of Taxol via an intramolecular Heck reaction. In addition, Morihira et al. (1998) reported the success of this approach to obtaining Taxol.

Although the successful chemical total synthesis of Taxol is a great achievement in the scientific community, it cannot be commercialized within the foreseeable future because the chemical synthesis needs more than twenty steps and the yield of several steps is very low. This makes it too complex and too expensive.

TAXOL FROM ENDOPHYTIC FUNGI

In 1993, Stierle et al. (1993) reported the first Taxol-producing fungus Taxomyces andreanae. Although the yield of Taxol is only as low as 24-50 ng/l, this finding causes scientists' great interest. Ever since, there have been a few reports on the isolation of Taxol-producing endophytic fungi (Strobel et al., 1996; Li et al., 1996; Wang et al., 2000), demonstrating that organisms other than Taxus species could produce Taxol. Thus, fermentation processes using Taxol-producing microorganisms may be an alternative promising way to produce Taxol.

Meanwhile, the biggest problem of using fungi fermentation to produce Taxol is its very poor yield and unstable production. The Taxol yield of such reported fungi varies from 24 ng to 70 µg per litre culture (Stierle et al., 1993; Strobel et al., 1996). One strain of Pestalotiopsis microspor CP-4 (Li et al., 1996) produces Taxol varying from 50 to 1487 ng/l, indicating that it is genetically unstable. To solve such a problem, current studies mainly focuses on the tedious work of finding and isolating fungi with high, stable yield of Taxol, as well as optimization of fermenting conditions like that of Taxus cell cultures.

Although the amount of Taxol produced by most endophytic fungi associated with Taxus trees is relatively small when compared with the trees, the short generation time and high growth rate of fungi make it worth while to continue our investigation of these species. After nearly two years work, our research group has isolated a few endophytic fungal strains from *Taxus chinensis var. mairei and Taxus yunnanensis.* One strain of *Ozonium* species BT2 can produce both Taxol and taxane baccatin III, an important intermediate for Taxol (unpublished), and optimization of fermenting conditions on this fungus is under process.

ENGINEERING FOR TAXOL PRODUCTION

As we have stated above, the Taxol biosynthetic pathway has been basically elucidated but for a few undefined steps. Once this pathway is fully understood, we will be able to bioengineer it to produce more Taxol and less of the unwanted compounds. Promoting over-expression or suppression of chosen genes to increase Taxol yields and simplify the purification process can do this. In 2001, Huang et al. (2001) reported biosynthesis of taxadiene, the key intermediate of Taxol, by over-expressing genes encoding isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase and taxadiene synthase in cell-free extracts of *Escherichia coli*. In addition, by the expression of three genes encoding certain enzymes on the terpene biosynthetic pathway in a single strain of *E. coli*, taxadiene can be conveniently synthesized in vivo, at the unoptimized yield of 1.3 mg per liter of cell culture. The success of both *in vitro* and *in vivo* synthesis of taxadiene bodes well for the future production of taxoids by non-Taxol producing organisms through pathway engineering. Recently, Jennewein et al. (2005) reported that coexpression of *Taxus* cytochrome P450 reductase with cytochrome P450 oxygenases involved in Taxol biosynthesis in yeast, demonstrating that functional transgenic coupling of the Taxus reductase with a homologous cytochrome P450 taxoid hydroxylase represents an important initial step in reconstructing Taxol biosynthesis in a microbial host.

Engineering for Taxol production either in Taxol-producing or non Taxol-producing organisms is a potential way in the future. But except for a few intermediate, there is no report of the final product of Taxol gained by using this approach.

CONCLUSION

In contrast to the booming pharmaceutical market of Taxol, the state of its natural resources, Taxus tree, is going from bad to worse. Therefore, careful use of this resource must to, while other approaches to Taxol sourcing should be emphasized and other in-depth research.

Taxol used in clinic and scientific research is mainly isolated from Taxus tree with skilled extraction procedures. Plant cell cultures and fungi fermentation provide extremely potential way to industrialize Taxol production if the bottlenecks of unstable and low yield could be broken in the years to come. Chemical synthesis may be the ultimate method to satisfy the urgent need of Taxol. It opens a pathway for the production of both the natural product itself and a variety of designed taxoids, which can be modified and may have strong effective activities. Research into the synthesis of Taxol is still ongoing with a number of groups (such as Magnus researchers at Austin, Texas; Wender's group at Stanford) around the world carrying out work in order to develop newer and shorter routes to this natural product, but also with a view to creating a range of structures based on Taxol but which may be more biologically active and/or easier to synthesize. Just recently, Ballatore et al. (2005) reported a general proto-

col for the synthesis of Taxol C-10 carbamates. The method is effective for the synthesis of Taxol C-10 derivatives, including bifunctional molecules, which can be designed and used to improve the overall biological profile of Taxol by linking the taxane skeleton to an auxiliary molecule.

In the present as well as in future studies, different research fields should be combined and collaborative groups need to be developed with the aim of boosting the yield of Taxol so as to drive down the anticancer drug price and satisfy the increasing demand of clinical and scientific research. The techniques of cell culture and fungi fermentation should be improved, cell lines or fungus colonies with high and stable content of Taxol should be isolated and more key breakthroughs are needed in the biological and chemical synthesis. As long as scientists' fundamental research and collaboration continue, the industrial production of Taxol could be achieved in the near future.

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